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IMPACT OF DROUGHT ON STREAM ECOSYSTEM STRUCTURE AND FUNCTIONING

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Abstract

Climate change is projected to increase the frequency and severity of extreme events, adding to the plethora of existing pressures that streams and rivers already face. Compound events such as drought may comprise numerous stressors that occur in concert to elicit ecological change. However the causal mechanisms of such impacts remain unknown, and research attempting to disentangle impacts of compound events, or link effects across levels of ecological organisation, remains in its infancy. This research investigates impacts of key drought stressors – sedimentation, dewatering and warming – across multiple ecological, hierarchical levels. At the individual level, macroinvertebrates displayed differential thermal sensitivity to warming which may explain idiosyncratic ecological responses reported elsewhere, whilst sedimentation intensified predator-prey interactions. Mesocosms were effective tools for studying drought stressors independently and in combination at the community and functional level. Dewatering main effects reduced the density of a common taxon and functional feeding group biomass, whilst all three stressors sometimes interacted together in complex ways. Stressors also had quantifiable effects at the whole-system level, e.g. stream metabolism. This study provides initial findings pertaining to drought impact causative mechanisms across multiple levels of ecological complexity, highlighting the importance of an experimental approach to predict future effects of compound events.

For Mum and Dad

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“*And from his alder shades and rocky falls, And from his fords and shallows sent a voice*”

The River Derwent, William Wordsworth

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CHAPTER ONE

Introduction

Extreme events are key elements of the natural variability in Earth's climate and include hydrological extremes such as floods and droughts and, in regards hydrological extremes, are defined as "*a rare or unusual weather or climatic occurrence… and/or the extreme physical phenomena in river catchments"* (Ledger & Milner, 2015). Climate change is expected to alter patterns of streamflow across the globe (Kundzewicz *et al*., 2008), with increasing heavy precipitation exacerbating flood events in some regions (Kundzewicz & Kaczmarek, 2000), and rainfall deficits leading to long term hydrologic droughts elsewhere (Burke *et al*., 2010; Dai, 2012). At the U.K. scale, altered rainfall patterns are projected to increase winter floods in Scotland and supraseasonal droughts in south east England (Vidal & Wade, 2009), leading to increases in river flow variability (Watts *et al*., 2015) and the latter predicted to increase the occurrence of hydrologic drought in lowland U.K. streams (Whitehead *et al*., 2006). Future climate change is thus likely to increase both the severity and frequency of these extremes (IPCC, 2012), and lead to unprecedented events with potentially devastating ecological consequences (Ledger & Milner, 2015) (although longer term effects can have evolutionary implications (Douglas *et al*., 2003; Milner *et al*., 2013) resulting in positive outcomes – e.g. selection of biotic resistance and resilience traits and adaptation to flow extremes (Bonada *et al*., 2007; Lytle & Poff, 2004)). Of the two hydrological extremes mentioned, least is known about drought impacts on freshwater environments (Lake, 2011) and there is an urgent need to gain a strong mechanistic understanding of these events in order to manage and mitigate their possible short-term future effects.

Although floods can occur rapidly with no prior meteorological extremes, drought often develops slowly, commencing as a meteorological drought and rainfall deficit before effects resonate through to soil moisture and groundwater hydrology (Van Loon, 2015). In future, the integrity of both terrestrial and aquatic systems may be threatened by an increased incidence of drought. Stream and river ecosystems however are particularly vulnerable (Woodward *et al.*, 2010) to extreme events (Vörösmarty *et al*., 2010; Heino *et al*., 2009) since they contain a disproportionately high species richness relative to global surface area (Dudgeon *et al*., 2006), and rely on and are structured by hydrological conditions in the surrounding catchment (Hynes, 1975). Drought effects on freshwater ecosystems could also have profound impacts on human society, including the reduction of available water for public supply (Delpla *et al*., 2009; Mosley, 2015). The impacts of hydrological drought on aquatic ecology can be exacerbated by anthropogenic pressures such as water withdrawals (Lake, 2011) which have increased sharply in recent years (Strayer & Dudgeon, 2010), as well as hydromorphological river modifications and poor water quality (Dudgeon *et al*., 2006; Hille *et al*., 2014; Jones & Byrne, 2010; Whitworth *et al*., 2012). As such the impetus to understand how drought affects running water systems has never been greater.

Drought can be regarded as a stepped and sequential disturbance event, with several critical stages occurring between drought formation and termination (Boulton, 2003). A disturbance can be defined as "*any relatively discrete event in time that disrupts ecosystem, community or population structure and changes resources, substrate availability or the physical environment*" (Pickett & White, 1985), whilst Resh *et al*. (1988) controversially adds a 'predictability' clause such

that only those events that occur *unpredictably* constitute a true disturbance. During the formation of a drought, a reduction in flow can result in entrained sediment particles, if present, being deposited on the river bed (Schalchli, 1992) and a loss of water depth and pelagic habitat (UKTAG, 2013). Further reductions in depth causes lateral disconnection of the river from the riparian zone (Bogan *et al*., 2015), and lowering of the water table can weaken vertical movement to hyporheic refugia (Lake, 2003) owing to both drying and anoxia of the hyporheos (Boulton & Stanley, 1995; Smock *et al*., 1994), though the hyporheic zone has also been shown to be an important refuge during drought (e.g. Stubbington *et al*., 2015). As drought develops, flow cessation occurs, water level falls sharply, and sections of river bed (e.g. riffles) become exposed such that any remaining wet habitat forms a series of disconnected pools (Caruso, 2001). These pools may provide refugia for aquatic biota from desiccation (Lake, 2003; Taylor, 1997). Following the fragmentation of the bed, marked changes in water quality and temperature occur (Drummond *et al*., 2015; Verdonschot *et al*., 2015) and biotic interactions may intensify (Dollar *et al*., 2003). Pools shrink with the evaporation of remaining water until completely dry (Boersma *et al*., 2014; Tramer, 1977). Fig. 1.1 summarises these temporal changes that occur in running waters during drought, illustrating the increasing stress as the event develops.

To date, hydroecological drought research has focused primarily on macroinvertebrate community structure and measurable attributes such as species richness, total abundance and community composition (Dewson, *et al*., 2007a; Wright *et al*., 2004), as well as other biotic indices including the percentage of Ephemeroptera, Plecoptera and Trichoptera taxa (% EPT; Calapez *et al*., 2014).

Frequently drought results in a reduction in macroinvertebrate richness (Extence, 1981) and altered composition (Cowx *et al*., 1984) whilst density effects are less clear (Dewson *et al*., 2007a; Lake, 2011b; Ledger *et al*., 2012). Fish can often be highly sensitive to drought stress, especially salmonids which often reduce in density as water temperature increases and dissolved oxygen concentration is reduced (Brooker *et al*., 1977; Cowx *et al*., 1984). An increase in benthic fish densities such as bullhead (*Cottus gobio*) can sometimes occur at the expense of pelagic drift-feeding fish species (Elliott, 2006) highlighting how drought can alter the competitive relationship between common fish taxa. Macrophytes have been shown to be particularly sensitive to flow regime (Hearne & Armitage, 1993) and sedimentation (Wood & Armitage, 1999), with drought reducing macrophyte cover (Wright & Symes, 1999) and stream drying resulting in the replacement of aquatic species with terrestrial herbs (Holmes, 1999; Westwood *et al*., 2006). Most recent hydrologic drought studies have assessed the ecological consequences arising directly from abiotic stress, but few have attempted to disentangle the ecological effects arising from drought multiple stressors, nor to understand their mechanistic basis. Likewise, most studies have focused primarily on structural assemblage change in response to drought, yet whether functional processes are equally affected remains largely to be explored. In addition, few studies have considered effects across multiple levels of ecological complexity, resulting in a myriad of crucial findings yet to be fitted together, in order to give a complete story of how drought affects the ecology of running waters. In particular, a growing number of research studies have taken a macroecological approach to determine the movement and distributional constraints of biota at large spatial scales in response to a growing

threat from climate change. However the relevance of these studies in the field of freshwater ecology may be limited, as aquatic taxa are more constrained within the boundaries of their habitat. Therefore, determining the resistance of biota and associated ecological processes to global change may provide an insight into future ecological responses as biota are forced to tolerate environmental stress, or perish (Woodward *et al*., 2010). Fig. 1.2 summarises these knowledge gaps in the ecological drought impact literature to date.

Hydrologic droughts can be unpredictable and infrequent phenomena, and research on these events is inherently challenging (Humphries & Baldwin, 2003). Studies of drought are often fortuitous and typically begin after the onset of the event itself, and thus lack pre-drought baseline data with which to evaluate impacts (e.g. Wright *et al*., 2004; but see Matthews *et al*., 2013). Furthermore survey approaches are often confounded by extraneous environmental variation (Harris *et al*., 2007) and lack predictive power (Stewart *et al*., 2013). Whilst temporary river (predictable annual drying) studies have been used to predict impacts of drought in perennial systems (Leigh *et al*., 2015; e.g. Westwood *et al*., 2006), there is a risk that these systems are at different ecological equilibrium to permanent flowing systems as communities adapt (e.g. greater proportion of resistance traits corresponding to small size, aerial dispersion and respiration (Bonada *et al*., 2007; Leigh *et al*., 2016)) to cope with the regular and predictable stress (Humphries & Baldwin, 2003; Larned *et al*., 2010; Stubbington *et al*., 2009). Experimental drought studies can offer an alternative approach, by mimicking drought effects in artificial permanent streams in order to make predictions on the effects of natural drought scenarios in future. What is more, such approaches are able to identify causal mechanisms by

controlling for confounding variables whilst manipulating others (Thompson *et al*., 2013) and have proven successful in climate change research (Fordham, 2015).

Figure 1.1. **Critical stages of drought.** Left hand diagrams depict longitudinal pool-riffle stream profiles and right hand diagrams depict lateral stream profiles with sloped banks. N.B dashed box illustrates the focus of this thesis.

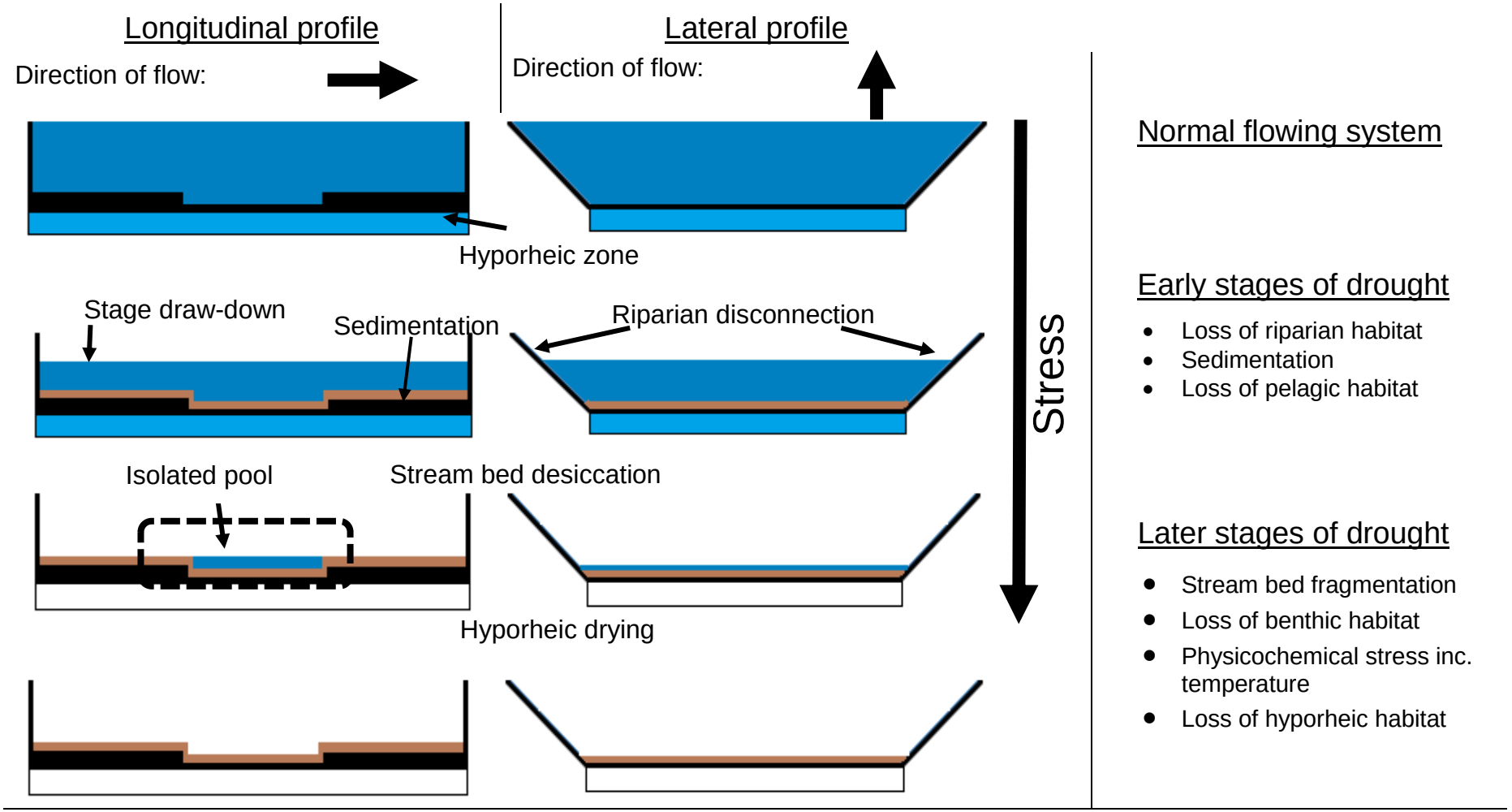
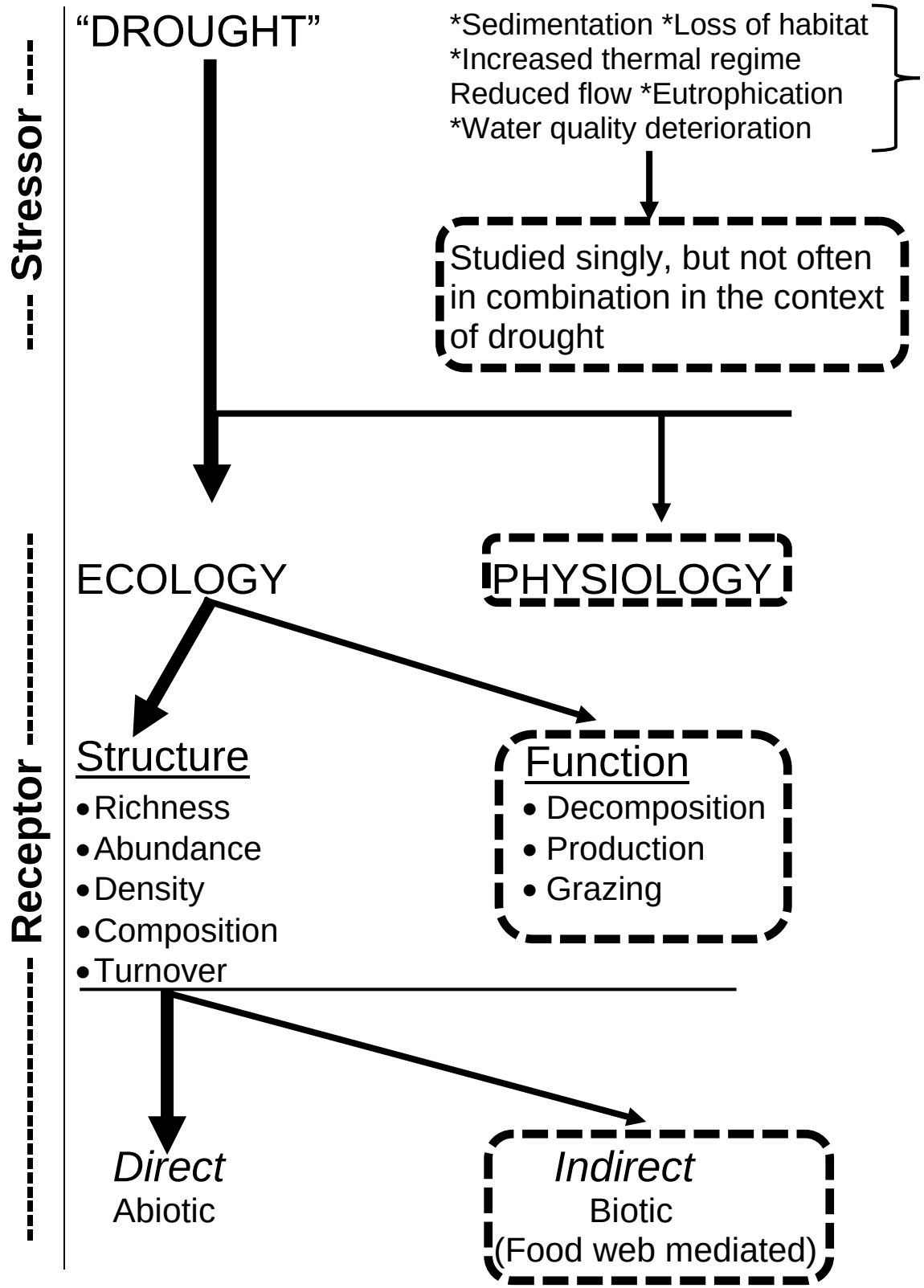


Figure 1.2. **Conceptualisation of drought research to date.** Whilst hydrologic drought is a multitude of stressors acting simultaneously, most studies are unable to disentangle the mechanistic basis. Dotted boxes illustrate areas requiring further investigation. Arrow thickness (not to scale) denotes degree of research focus to date.



1.1 LOWLAND CHALK STREAMS AS MODEL SYSTEMS SUSCEPTIBLE TO DROUGHT

In order to assess the ecological effects of drought in running waters using an experimental approach, a suitable river system must be justifiably selected on which to base the research's methodology. A number of important considerations are needed, including contextual and ecological relevance of different river systems both now and in future when the climate has changed, in order to ensure the most relevant data is obtained that can subsequently inform water managers.

Chalk streams are unique and ecologically rich river systems (Wright & Symes, 1999) which occur internationally; however approximately 85% of these quintessential rivers exist within England, primarily along a north east band from Dorset to the Norfolk Broads (The Wildlife Trusts, n.d.; Hampshire Biodiversity Partnership, 2000). Chalk streams occur only where cretaceous chalk, a highly porous rock that allows rainwater to percolate readily to the underlying aquifer, is at the Earth's surface. When the aquifer rises to the surface, it flows through springs (Berrie, 1992), contributing ~80% of total annual water input (Wood & Petts, 1999). As groundwater slowly percolates through chalk, it drains into streams and rivers providing constant flow during periods of dry weather (Bradford, 2002). As such, these systems are characterised by stable hydrological and physico-chemical parameters (Webb, 1996; Webb & Zhang, 1999), providing suitable habitat for rare taxa such as the southern damselfly (*Coenagrion mercuriale*) and the fine-lined pea mussel (*Pisidium tenuilineatum*) (Hampshire Biodiversity Partnership, 2000). These nutrient rich, stable and clear water stream systems (Fig. 1.3) typically contain large dense macrophyte beds including the ecosystem engineer species, water crowfoot

(*Ranunculus* spp.), and support high densities of moderate to high flow preference fish species including brown trout and bullhead, in turn supporting terrestrial predators (Fig. 1.4). However, chalk streams are particularly susceptible to long term droughts owing to their reliance on groundwater input, leading to the cessation of flow following a lack of winter aquifer recharge.

In order to address the research gaps identified in Fig 1.2, and owing to the difficulties associated with studying natural drought as outlined above, an experimental approach was undertaken using chalk streams as model systems. Although limited in number, chalk streams were chosen for the following reasons:

1. These river systems are of high conservation importance as they contain endangered and biodiversity action plan (BAP) species.

2. Chalk streams are hydrologically stable systems vulnerable to climate change and extreme events.

3. Within the U.K., chalk streams fall within the geographic area predicted to experience an increase in supraseasonal droughts (Vidal & Wade, 2009).

4. The stable temperature of groundwater which constitutes the majority of discharge in these systems provides an ideal opportunity for experimental thermal regime manipulations in the field and / or laboratory.

Figure 1.3. **Photographs of chalk streams near to the mesocosm facility in Hampshire, U.K.** a) Candover Brook near Abbotstone (51°10’8”N, 1°19’10”W) and b) River Itchen near Ovington (51°08’4”N, 1°19’6”W). Photographs taken between 2012 and 2015 (Source: Gavin Williams).

a)

b)

Figure 1.4. **Photographs of iconic chalk stream flora and fauna**. a) water crowfoot, *Ranunculus* sp.; b) brown trout, *Salmo trutta*; c) bullhead, *Cottus gobio* and kingfisher, *Alcedo atthis.* Photographs taken between 2012 and 2015 (Source: Gavin Williams).

a)

b)

c)

d)

This research takes a novel approach in investigating the ecological effects of individual stressors that combine during periods of hydrologic drought across multiple levels of ecological complexity. Research is focused on three key drought stressors: dewatering (leading to a loss of water volume, pelagic and benthic habitat), sedimentation, and warming.

1.2 ECOSYSTEM FUNCTIONING

Ecosystem functioning refers to the processes that occur within streams which lead to the provisioning of ecosystem services (Millenium Ecosystem Assessment, 2005; Naeem *et al*., 1999) such as provisioning of drinking water, provisioning of fisheries, and carbon sequestration. Functioning often refers to the rates of flux, e.g. rate of production within a system (e.g. Ledger *et al*., 2011). Rates of key processes cannot be readily determined from community structure, because a multitude of factors including species redundancy and identity can confound simple correlation relationships between biodiversity and ecosystem functioning (Cardinale *et al*., 2002; Dangles & Malmqvist, 2004; Jonsson *et al*., 2002; Vaughn *et al*., 2007). Furthermore it has been found that structure and function can vary in sensitivity to stress (Magoulick, 2014), and thus both should be measured to develop a complete understanding of drought ecological effects (Death *et al*., 2009). Climate change and extreme climatic events will increase societal demand for running water ecosystem services (i.e. drinking water; Eigenbrod *et al*., 2011) whilst threatening to reduce the importance and availability of services provided (Kundzewicz *et al*., 2008; Terrado *et al*., 2014). There is currently a lack of knowledge of drought impacts on functioning, and an urgent need to develop research programmes to address this.

1.3 DROUGHT AS A COMPOUND DISTURBANCE

Drought can be regarded as a compound disturbance comprising multiple stressors (e.g. sedimentation (Wood & Petts, 1999); habitat fragmentation (Bogan & Lytle, 2011); warming (Galbraith *et al*., 2010; Van Vliet *et al*., 2011) and acidification (Bowman *et al.*, 2006)) that may combine to produce the drought event. Here, stressors are defined as "*a variable that potentially provokes a measurable biological or ecological response*" after Statzner & Bêche (2010). Climate change is expected to increase the frequency and co-occurrence (Leigh *et al*., 2015) of extreme event stressors, yet research quantifying their impacts within river systems remains in its infancy (Nõges *et al*., 2016). In order to understand the mechanistic basis of drought events, drought must be broken down into its constituent stressors (Statzner & Bêche, 2010) and their singular and in-combination effects assessed. Of the realm of stressors that may occur during hydrologic drought, the following three stressors are explored in this research: sedimentation, dewatering, and warming.

1.3.1 SEDIMENTATION

Sedimentation can be a common occurrence during drought as fine particles are deposited onto the stream bed with declining flows. Sedimentation is likely to worsen in future as a result of increased demand for land to produce greater agricultural output (Strayer & Dudgeon, 2010). In southern England, 94% of sediment accumulating in streams is from land surface sources (Walling *et al*., 2003) with large quantities ending up within chalk streams (Walling & Amos, 1999). Sedimentation is known to increase macroinvertebrate drift (Larsen & Ormerod,

2010; Piggott *et al*., 2015; Dewson *et al*., 2007b), smother and damage macroinvertebrate (Lemly, 1982) and fish (Wong *et al*., 2013) respiratory structures, reduce egg hatching success of macroinvertebrates (Kefford *et al*., 2010) and fish (Soulsby *et al*., 2001) and invoke fish embryo deformities (George *et al*., 2015), directly bury macroinvertebrates (Wood *et al*., 2005; Wood *et al*., 2001), and reduce light availability and alter habitat structure and quality for macrophytes (Jones *et al.*, 2012). Furthermore, sedimentation can reduce feeding by fishes (Greer *et al*., 2015), smother [and reduce the organic content of] biofilm resources for macroinvertebrates (Graham, 1990), and can clog interstitial spaces (Wood & Armitage, 1997), reducing oxygen exchange (Jones *et al*., 2015) and impeding vertical macroinvertebrate movement (Vadher *et al*., 2015), and may ultimately modify the benthic habitat of the stream bed. However, our understanding of how sediment indirectly affects animals (i.e. modified species interactions mediated by altered benthic habitat), how important sedimentation during drought is relative to other stressors, and whether or not sediment may interact with other stressors during drought, all remain poorly understood.

1.3.2 DEWATERING

As stage decreases, the three dimensional space in which animals can move is reduced, thus reducing available pelagic habitat and movement within the water column for many fish species. Furthermore, as water disconnects from the riparian zone, and as riffles become exposed, available benthic habitat is lost, forcing benthic macroinvertebrates and fish (e.g. *Cottus gobio*) into increasingly confined space (Covich *et al*., 2003; Lake, 2011a). The degree of drying and the size of the remnant isolated aquatic habitats can determine community composition and

extinction risk (Miyazono & Taylor, 2013; Love *et al*., 2008). Refugia for macroinvertebrates during drought include isolated pools (Bond *et al*., 2008; Covich *et al*., 2003), the hyporheic zone (Stubbington *et al*., 2015), and areas able to remain damp, e.g. beneath woody debris (Golladay *et al*., 2004). Fish may be able to seek refuge from drought for short periods, particularly in deeper isolated pools (Elliott, 2000) The ability of taxa to mobilise during drying will determine the likelihood of them locating refugia (Gough *et al*., 2012), thus determining the density of taxa within such refugia as drought develops (Covich *et al*., 2003). Large bodied predators and other vulnerable taxa are likely to become extirpated (Jellyman *et al*., 2014) following dewatering, whilst biotic interactions between surviving predators and prey within pools may intensify (Dollar *et al*., 2003). However the ability of predators to shape the remnant community within remaining refugia such as isolated pools has not been quantified, and it remains unknown how important dewatering *per se* is, relative to other drought stressors. Furthermore, whether or not dewatering interacts with other drought stressors is unknown.

1.3.3 WARMING

In future, water temperature will increase in line with rising atmospheric temperature (Webb & Zhang, 2004; Chessman, 2009; Durance & Ormerod, 2007; Hannah & Garner, 2015). A mean temperature increase is predicted to play a leading role in shaping freshwater biodiversity (Mantyka-Pringle *et al*., 2014) and ecosystem functioning (Perkins *et al*., 2010; Dang *et al*., 2009). Warming has been shown to increase macroinvertebrate density (Friberg *et al*., 2009) and to positively correlate with fish density (Friberg *et al*., 2009) and size (O'Gorman *et al.*, 2012), whilst water temperatures greater than upper thermal tolerances may reduce habitat availability

for salmonids (Keleher & Rahel, 1996) and determine fish distribution (Dunham *et al*., 2003), as well as to reduce the reproductive success of benthic fish such as *Cottus gobio* (Dorts *et al*., 2012). The importance of temperature as a macroinvertebrate structuring mechanism has also been recently evidenced by Hill & Hawkins (2014), with the macroinvertebrate community composition reflecting both their thermal optima and the water temperature. Macrophyte growth may also increase with warming in deeper waters (Rooney & Kalff, 2000) whereas contrasting effects are most likely in shallower waters which instead turn eutrophic (McKee *et al*., 2003). Warming may also have contrasting effects at different levels of ecological complexity (i.e. reduced community biomass but increased individual growth rate, (Cross *et al*., 2015)). Water temperature can be particularly sensitive to atmospheric warming during drought (Van Vliet *et al*., 2011; Velasco & Millan, 1998), since the thermal capacity of the water is reduced (Larned *et al*., 2010; Elliott, 2000). For example, a 95% reduction in pool water volume has been demonstrated to increase temperature range from 10-17 °C to 8-35 °C (Drummond *et al*., 2015). Additionally, heatwaves, hot days and droughts are likely to occur in synchrony more frequently in future (Galbraith *et al*., 2010) increasing the potential severity of stream water temperature maxima, and continued riparian deforestation may too enhance stream water temperature in future (Bowler *et al*., 2012), elevating temperatures beyond the thermal tolerances of biota (Broadmeadow *et al*., 2011). Whilst it has long been known that thermal physiology can in part explain ecology, e.g. population abundance (Cowles & Bogert, 1944), forging a formal link between these fields remains challenging (Gaston, 2009). In the present context, questions remain as to whether thermal physiological thresholds of stream biota underpin

observed shifts in community structure during drought. Again, the importance of temperature relative to other stressors is unknown, along with whether or not temperature may interact with other stressors in order to determine community structure and functional impacts.

1.4. THESIS OVERARCHING AIMS

Building on existing drought research, the aim of this research was to expand the boundaries of existing drought impact knowledge. Specifically, this thesis aimed to:

1. Determine the underpinning mechanistic basis of hydrological drought effects (i.e. which stressors are more pervasive and whether stressors interact)

2. Determine if and how drought pressures lead to effects at multiple levels of ecological complexity (i.e. determine effects from individual to whole system).

In order to achieve these aims, three principal objectives were set:

- Investigate drought stressors in isolation and in combination in order to assess both the main effects and interaction effects of stressors on macroinvertebrates, macrophytes, fish (sediment main effects only), and functional processes.
- Explore how responses at the individual level (behavioural responses and physiological responses) may help to explain community level responses during drought on fish via predation and on macroinvertebrates via mortality.

- Examine whether individual and community level impacts (e.g. benthic community assemblage) from drought may have driven ecosystem functioning processes.

1.5. THESIS OUTLINE

This thesis is written partly in the form of extended papers and therefore some sections may be repeated among chapters.

Chapter two is the first of four consecutive data chapters. This chapter investigates sedimentation, dewatering and warming singly and in-combination on macroinvertebrate community structure. Stressor main effects vs. interaction effects are compared, as are the effects of single and compound stressor treatments on community structure. Sedimentation is found to be the most pervasive drought stressor, whilst warming effects are present in all significant interactions. Community changes were found to be solely attributable to population densities, and evidence for the drought resistance hypothesis is provided.

Chapter three explores how the aforementioned stressors affect, singly and in combination, key *functional* processes. Functioning is explored at a multitude of levels, from standing stock biomass to production to whole-stream metabolism. Fauna biomass follows density patterns from the previous chapter, macrophyte growth and photosynthetic capacity are shown to be particularly sensitive to the applied stressors, sediment is found to elevate benthic respiration and warming effects suggest a reduction in carbon sequestration capabilities of drought impacted streams. Effects at lower ecological levels (e.g. macroinvertebrate standing stock) do not appear to resonate to whole-system processes such as stream metabolism.

Chapter four explores whether drought mediated impacts on channel morphology may have indirect effects upon biota. Moreover, this chapter explores individual behavioural responses to abiotic drought stress. Findings illustrate that predator-prey interactions during drought may intensify top-down control, driving down prey population abundance, and suggest indirect mechanisms during drought may have previously been underestimated.

Chapter five explores macroinvertebrate physiology to determine whether drought may affect individual thermal activity thresholds such as CTmax and Heat Coma. A comparison of water temperatures during drought and non-drought conditions, alongside taxa physiological traits allows warming tolerances of taxa to be calculated, revealing that a greater proportion of the macroinvertebrate community may cease functioning during drought compared to non-drought periods, owing to exceedance of physiological thresholds. Evidence that respiratory mode partly determines the CTmax of macroinvertebrates is presented.

Chapter six brings the individual thesis chapter's conclusions and key research outcomes together in an overarching discussion. A special focus is given on how the findings inform river restoration practice in regards hydrological drought. Recommendations for further research are given to develop the research presented in this thesis, which would help mitigate future ecological structure and function drought impacts.

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CHAPTER TWO

Drought as a compound disturbance: Part 1

Community structure

2.1. ABSTRACT

Hydrological extremes such as droughts are likely to become more prevalent in running waters and research is needed to further understanding of their ecological consequences and mechanistic basis. Drought can be regarded as a compound disturbance event that consists of numerous stressors acting in concert. The effect of drought may depend on which stressors co-occur, and whether they interact. This chapter describes the results of a field experiment conducted in stream mesocosms to assess the ecological impact of three core stressors (sedimentation, dewatering and warming) that frequently co-occur during drought. The main effects of stressors and their interactions were determined using a 2 x 2 x 2 factorial design, with macroinvertebrates selected as key bioindicators of environmental stress (impacts on key ecological processes reported in Chapter 3). Stressor effects were detected at both the community and population level. A facilitative interaction between warming and sediment increased total macroinvertebrate density relative to controls when both stressors were combined, whereas an interaction (inhibition) between warming and dewatering significantly decreased total macroinvertebrate density when both were combined. Pairwise RDA models revealed that compound stress significantly explained 8.4-12.8% of community variance and demonstrated the overall deleterious effects of sediment. Pairwise effects incorporating temperature were frequent, highlighting the potential for unexpected compound events to become more frequent in future as global temperatures increases. This research provides the first known experimental test of drought stressor interactions, and illustrates the importance of compound stress during drought in shaping the macroinvertebrate community.

2.2. INTRODUCTION

Climate change is expected to alter global rainfall patterns (IPCC, 2013; Watts *et al*., 2015) with potentially profound consequences for hydrological regimes in rivers and streams (Burke *et al*., 2010; Prudhomme *et al*., 2012). Coupled climate-hydrology models predict that hydrological droughts will increase in both frequency and severity in future (IPCC, 2012) and such impacts are likely to be further exacerbated by anthropogenic pressures such as water abstraction (Bond *et al*., 2008). Short term seasonal droughts are projected to increase in frequency across the U.K. (Blenkinsop & Fowler, 2007), with supra-seasonal events expected to increase in frequency in south east England (Vidal & Wade, 2009). Research on the ecological effects of drought in running waters has increased in recent years, but understanding still lags behind that of other disturbances, especially flooding (Lake, 2003; Lake, 2011). In particular, the mechanistic basis of droughts which drive ecological changes are poorly understood.

To date, experiments investigating drought have focused mainly on 'drying' (e.g. Closs & Lake, 1996; Haag & Warren, 2008; Power *et al*., 2008; Wood & Petts, 1999a; Ledger *et al*., 2008). Whilst drying can have direct effects on community structure (e.g. reduced richness; Ledger *et al*., 2012) it can also cause sedimentation (Wright & Berrie, 1987) as particles fall out of suspension, and constrain ecosystem size (Dewson *et al*., 2007a). Drying can result in the formation of isolated pools (Bogan & Lytle, 2011; Bonada *et al*., 2006; Chester & Robson, 2011; Nhiwatiwa *et al*., 2009; Robson & Matthews, 2004) which may increase variation in water temperature (Galbraith *et al*., 2010), reduce dissolved oxygen (Elliott, 2000), increase conductivity (Beche *et al*., 2009) and modify pH (Drummond

et al., 2015). Macroinvertebrates may utilise isolated pools as refugia (Reich & Lake, 2015), although many taxa are extirpated as abiotic stress increases (Verdonschot *et al*., 2015). A lack of physical habitat may also reduce habitat heterogeneity and drive down overall richness (Cazaubon & Giudicelli, 1999).

The number of drought studies has grown in recent years, yet the causal mechanisms (i.e. specific stressors) that underpin ecological effects remain poorly understood. Multiple stressor studies in the wider literature are numerous, but many have focused on the impacts of toxins and agricultural stressors, not drought. Studies on toxins are particularly common, e.g. insecticides with herbicides (Boone & James, 2014), pesticides with pathogens (Buck *et al*., 2012), metal pollutants (Charles *et al*., 2006; Doroszuk *et al*., 2007), flow with pharmaceuticals *(*Corcoll *et al*., 2014) and metals with temperature (Pandolfo *et al*., 2010). Studies of agricultural stressors have investigated sediment with herbicides (Magbanua *et al*., 2013), sediment with nutrients *(Piggott et al*., 2015; Townsend *et al*., 2008; Wagenhoff *et al*., 2012) and sediment, nutrients and abstraction (Matthaei *et al*., 2010). Although drought can be viewed as a single stressor (i.e. a 'reduction in flow'; e.g. Magoulick, 2014), these events generate a range of physical and chemical conditions (e.g. sedimentation, water and habitat loss, increased temperature and conductivity, reduced dissolved oxygen) that may or may not interact in complex ways to cause ecological change (Statzner & Bêche, 2010).

Drought effects may depend on whether or not specific stressors co-occur, and interact. Many stressors have been studied in other environmental contexts, often singly or in pairs. For instance, sedimentation studies have focused specifically on the effect of clogging and macroinvertebrate burial (Ciesielka & Bailey, 2001; Bo *et*

al., 2007; Chandrasekara & Frid, 1998; Wood *et al*., 2005; Wood *et al*., 2001; Kefford *et al*., 2010). Sedimentation can decrease overall macroinvertebrate abundance, trigger increases in abundance of opportunistic taxa e.g. Chironomidae spp. and reduce animal egg hatching success. Sediment can also alter predation risk (Clark *et al*., 2013; Martin *et al*., 2012), increase macroinvertebrate drift (Larsen & Ormerod, 2010) and constrain the vertical movement of macroinvertebrates within the stream bed (Mathers *et al*., 2014). Whilst survey studies show species have widespread sensitivity to sedimentation (Extence *et al*., 2013) typically sedimentation elicits general negative effects on community structure (Piggott *et al*., 2015) and reduces species richness of macroinvertebrates (Couceiro *et al*., 2011; Ramezani *et al.*, 2014).

Water level decline and associated reductions in the size of the benthic habitat can limit the abundance of large predators within streams and rivers (Jellyman *et al*., 2014), determine the size and length of aquatic food webs (McHugh *et al.*, 2015), alter predation pressure (Nhiwatiwa *et al*., 2009), divide populations and reduce productivity (Stanley *et al*., 1997), and temporarily increase (Dewson *et al*., 2007a) or decrease (McIntosh *et al*., 2002) taxa densities. Temperature has been widely studied, from the individual level (e.g. organism thermal tolerance; Dallas & Rivers-Moore, 2012), to the community level (O'Gorman *et al*., 2014). At the individual level, temperature can determine the metabolic rate (Gillooly *et al.*, 2001), growth rate (Pockl, 1992; Sutcliffe *et al*., 1981) and feeding rate (Maltby *et al*., 2002) of biota. Temperature can also shape entire stream communities as evidenced for example by work in geothermal Icelandic streams (Woodward *et al*., 2010). High temperature can exceed the physiological tolerance limits of organisms and cause

mortality (Bailey, 1955; Mundahl, 1990). Piggott *et al*. (2015) revealed that warming can have negative effects on macroinvertebrate assemblages, such as reduced taxa abundances and increased drift propensity. Yet not all studies reveal similar responses – e.g. no effect (Dossena *et al*., 2012) – indicative of context dependent responses. Moreover, warming can reduce dissolved oxygen availability through reduced supply and increased metabolic demand (Ficke *et al*., 2007; Verberk *et al*., 2011), resulting in mortality in taxa that possess a limited ability to regulate intake (Verberk & Bilton, 2013; Verberk & Calosi, 2012).

Persistence of biota depends on the capacity of individuals to withstand the cocktail of stressors in the local environment. Whilst the ecological effects of temperature, water loss and sedimentation have been tested singly or in pairs within other environmental contexts (e.g. agriculture; Piggott *et al*., 2015), the interactive effect of all three stressors is explored here for the first time. As synergism among stressors is predicted to increase extinction risk in future (Brook *et al*., 2008), gaining an understanding of how drought stressors interact will help water managers alleviate drought effects in future when the climate dries.

Droughts occur unpredictably in the U.K. and mesocosms have been advocated as a means to simulate these events at small spatial and temporal scales (e.g. Ledger *et al*., 2012; Woodward *et al*., 2012; Lancaster & Ledger, 2015). In particular, mesocosms are replicable (Harris *et al*., 2007) and can have realistic physicochemistry (Ledger *et al.*, 2008) and food web characteristics (Brown *et al*., 2011). This chapter reports the results of a 2 x 2 x 2 factorial mesocosm experiment designed to investigate the independent and interactive effects of warming, sedimentation and dewatering as key stressors occurring during droughts. Factorial

experiments can identify causal mechanisms (Downes, 2010) and are advocated for use in multiple stressor experiments. This experiment tested seven hypotheses: H_1 sedimentation will have negative effects on the macroinvertebrate community structure (specifically reduce taxa densities, cf. Piggott *et al*., 2015). H_2 sedimentation will be the most pervasive stressor (i.e. will elicit the greatest number of significant responses; Piggott *et al*., 2015b). H_3 warming will have negative effects on macroinvertebrate community structure (specifically reduce taxa densities, cf. Piggott *et al*., 2015). H_4 dewatering will decrease species richness due to a reduction in available habitat (Cazaubon & Giudicelli, 1999). H_5 dewatering will increase population densities in confined spaces (Dewson *et al*., 2007). H_6 the direction of taxon responses to stressors will vary because taxa that are resistant / intolerant to stressors will respond positively / negatively, respectively. H_7 combined stressor treatments would have the greatest effects on taxa owing to the added stress of compound events mediated by additive and or synergistic interactions (Crain *et al*., 2008).

2.3 METHODOLOGY

2.3.1 Study site

The research was conducted in a series of outdoor stream mesocosms located at Fobdown Farm (51°6'4"N, 1°11'13"W), a watercress production facility operated by Vitacress Ltd in New Alresford, Hampshire UK, over a period of 42 days (April 2014 – May 2014). Forty channels were sited in close proximity with the same photoperiod, on a gravel bed adjacent to the Candover Brook, a small chalk stream (Fig. 2.1). Each channel was constructed from a horizontally-cut ribbed twin-walled sewage pipe (Fig. 2.2) and measured 300 x 30 x 15 cm (LWH). The substratum

consisted of a layer of gravel sourced from the site and closely matched that of the Candover Brook stream bed. Benthic habitat depth heterogeneity was incorporated into each mesocosm (Fig. 2.3) by dividing the channel laterally into three equal lengths (1m length each, 3m length total) with top and bottom sections consisting of 5 cm substratum depth, and central section consisting of 1 cm substratum depth, providing deeper pools. Prior to treatment effects, water depth was ~10 cm above deeper substrate sections, and ~14 cm in the central section (where substrate depth was more shallow) among all channels. Groundwater (pH: 7.42; water temperature: 10.25 °C; conductivity: 963 µS) was supplied to a 220 L header tank which was subsequently gravity fed to individual mesocosms through 12.5 mm inlet valves.

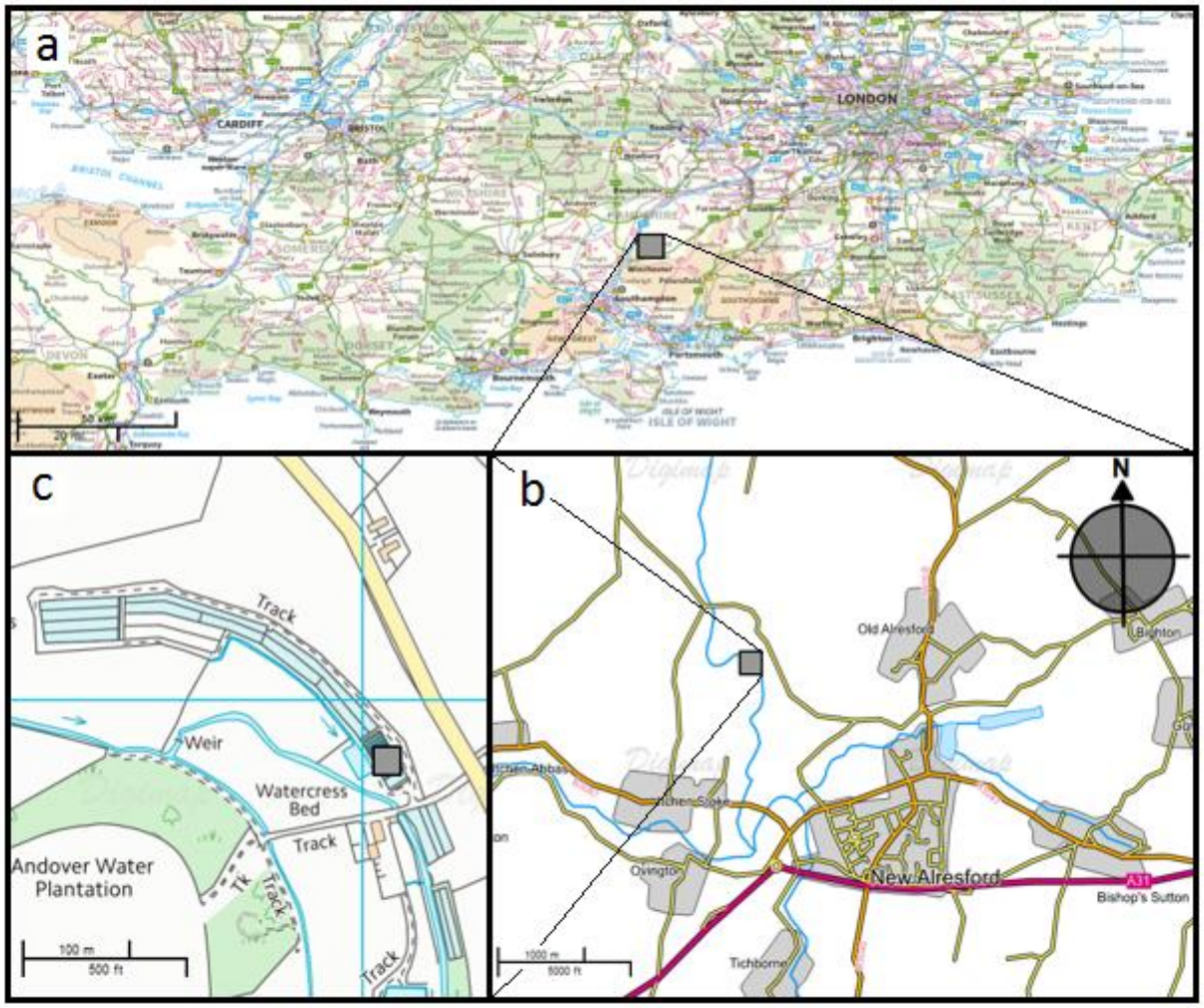


Figure 2.1. **Geographic location of the mesocosm facility**. Location within (a) Hampshire, southern England, (b) New Alresford, and (c) the watercress farm. © Crown Copyright and Database Right [2015]. Ordnance Survey (Digimap Licence).

Figure 2.2. **Photograph of mesocosms**. Taken shortly after construction (April 2014).

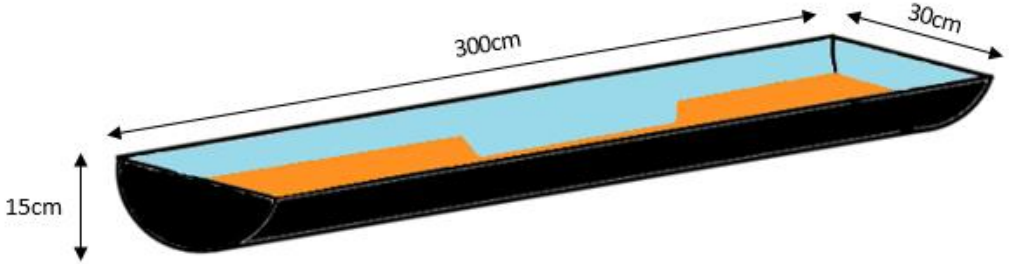


Figure 2.3. **Schematic diagram of a mesocosm channel**. Central orange colour illustrates incorporated channel depth heterogeneity

2.3.2 Experimental design

A 2 x 2 x 2 factorial experiment was conducted in the mesocosms, which were set up and allowed 25 days to establish. Three drought stressors – warming, dewatering, and sedimentation – were then applied singly and in combination (Table 2.1) on day 0, generating seven experimental treatments and a control. Each treatment or control was replicated five times, yielding 40 experimental units in total. Warming (Fig. 2.4) was achieved passively by isolation of water diverted from the header tank along an 18 m length of black pipe, and elevation of channels on blocks above the watercress bed. This technique produced a cooling effect at night (due to isolation of the raised channels from the water bath (watercress bed) beneath), resulting in a greater thermal regime as would be expected during drought. Sedimentation treatments received 2406.5 ± 148.5 g m^{-2} (dry weight) of fine sediment (Fig. 2.5a), obtained from a nearby stream and air dried for 14 days, by evenly distributing the material over the surface of the channels. Water loss was applied by reducing the depth of water over the substratum within pools to ~4.6 cm (63% decrease; Fig 2.5b), partially dewatering the raised sections of each channel. Terracotta tiles (24.1 cm^2; n = 1 per channel) were added to the centre of each channel on day 0 to calculate biofilm accrual m^{-2} following the experiment.

Table 2.1**. Summary table of treatment characterisation**. N.B. Codes in far left column are used throughout this chapter and chapter three for simplicity. C = control, D = dewatered, S = sediment applied, W = warmed.

Code	Temperature	Sedimentation	Water loss	Number of stressors
C	Not warmed	No sediment	Not dewatered	0
D	Not warmed	No sediment	Dewatered	1
S	Not warmed	Sediment	Not dewatered	1
SD	Not warmed	Sediment	Dewatered	2
W	Warmed	No sediment	Not dewatered	1
WD	Warmed	No sediment	Dewatered	2
WS	Warmed	Sediment	Not dewatered	2
WSD	Warmed	Sediment	Dewatered	3

2.3.3 Sample processing

Channels were seeded with macroinvertebrates, on day -1 following methods by Piggott *et al*. (2012), caught from the Candover Brook and an on-site feeder channel (Fig. 2.1). In short, this consisted of adding a standard load of macroinvertebrates to each channel to augment those naturally colonised from groundwater and oviposition, obtained by kick sampling with equal effort and randomly assigning samples to each channel. Benthic macroinvertebrates were left to colonise and sampled at the end of the experiment (day 42, 1 sample per channel) using a small Surber sampler (0.08 m^2) in the centre of each channel. This method follows after Piggott *et al*. (2012) and is a standard method used by (Ledger *et al*., 2012). Macroinvertebrates were subsequently preserved in 70% IMS and later sorted from debris and identified to the lowest practicable taxonomic unit (usually species). Chironomids were heated in 10% potassium hydroxide (KOH) solution at 60 °C for 15 minutes, then mounted onto slides with DMFH mountant and identified at x40 magnification using keys by Brooks *et al.* (2007) and Wiederholm (1983).

Water temperature was recorded continuously (TinyTag loggers, Gemeni Data Loggers Ltd, Sussex, U.K.) in each control (C; n = 5) and warmed (W; n = 5) channel to characterise temperature treatments. To determine physical abiotic factors that may explain biotic responses to treatments, maximum temperature and dissolved oxygen were recorded weekly in each channel (n = 40) (YSI proODO meter, YSI Ltd, Hampshire, U.K.) along with pH (day 42) using a YSI 6820 multi-meter (YSI Ltd, Hampshire, U.K). Additionally biofilm was scraped from terracotta tiles (day 42, 24.1 cm^2; n = 1 per channel) into 24 ml polypropylene bottles and stored in the dark ≤ -18 °C. 10 ml was subsequently oven dried, weighed, heated in a muffle furnace at 450 °C and reweighed to determine biofilm AFDM. A subsample of the dried sediment was taken to the laboratory and organic matter AFDM determined using a muffle furnace, as per above.

2.3.4 Data analysis

Response variable distributions were analysed using QQ plots, and outliers were examined using box plots. Normal distribution was statistically tested using Shapiro-Wilk tests and homogeneity of variance was tested using the Bartlett test.

Partial redundancy analysis (RDA) was conducted, due to binary short gradient variables, using CANOCO 4.5, to investigate macroinvertebrate community structure responses to treatment effects. Treatments were thus used as constraining variables, and dummy variables (categorical: 0, 1) were used to define treatments. Ordinations were conducted on square root transformed and proportions of total (i.e. standardised by sample norm) macroinvertebrate abundances after Ledger *et al*. (2006). A Monte Carlo permutation test (999 permutations) was used to determine whether explained variance of community structure was statistically significant (P <0.05) for each model. Additionally, pairwise

RDA models were used to compare macroinvertebrate community structure between the control and each treatment in turn, with the remaining six treatments entered as co-variables, thus removing their influence on the ordination axes. Taxa with > 20% explained fit to the model were used in constructing RDA bi-plots.

A three-way analysis of variance (ANOVA) was conducted to test for the main effect of each stressor, and their interactions, on macroinvertebrate community structure (richness, total density) and population structure (core taxon densities [i.e. present in >50% samples]). Biological data were log-transformed, if necessary, to improve normality and homoscedasticity, following methods by Townsend *et al*. (2008) and recommendations by Ives (2015). Bonferroni correction was conducted to reduce the number of type 1 errors, by dividing *P* (0.05) by the number of taxa tested (12) owing to the large number of tests conducted. A resultant *P* value of < 0.004 was used to determine if responses were significant. The ANOVA model tested for significance of individual stressors, and for the significance of interaction effects of stressors in combination.

Significant interactions detected by the three way ANOVA were subsequently followed up using Tukey HSD post-hoc tests to detect significant differences between treatment means. Three way ANOVA and Tukey HDS tests were conducted using R version 3.2.0.

2.4 RESULTS

2.4.1 Treatments

Experimental warming increased the mean, maximum, minimum and standard deviation of water temperature in the mesocosms (see Table 2.2; Fig. 2.4; Fig 2.6a). Warmed treatments (W) were on average 2.8 °C warmer than control (C) channels (mean day-time temperature). Warmed treatments had a greater day time maximum

(+5.8 °C) and a cooler night time minimum (-3.3 °C) than control (C) channels over the logging period (42 days), reflecting a more extreme thermal regime. Greater variability within treatments occurred during the day, compared to night time water temperatures. Fine sediment, which comprised 20.13 ± 2.53 % organic matter, evenly smothered the substratum. In addition to a reduction in water depth of 63% in (central) shallow substrate sections and 97% in deeper substrate sections (top and bottom end), dewatering also decreased the longitudinal wetted area by 60.2%. Treatments had no obvious effect on dissolved oxygen (11-15 mg^{-1} l Fig. 2.6b) or pH (7.5-8.5, Fig. 2.7).

Table 2.2. **Summary statistics of water temperature in the experiment**. Comparison of warmed (W) and control (C) treatments. Data are mean, max and min temperature values averaged from the permanent loggers over the duration of the experiment. Note: day and night determined as 09:00-20:59 and 21:00-08:59 respectively.

	W		**C**	
	Day	Night	Day	Night
Mean temperature (°C)	15.12	10.10	12.32	10.10
Standard Deviation	3.58	1.80	1.57	0.51
Maximum temperature (°C)	27.46	18.81	21.69	13.36
Minimum temperature (°C)	5.48	4.78	9.74	8.05

Figure 2.4. **Mean diel water temperature in the experiment**. Comparison of temperature time series (mean temperature for each time step, averaged across five replicates for each treatment) between control (C) and warmed (W) treatments for the period 29th April – 8th June, 2014. C = control, W = warmed.

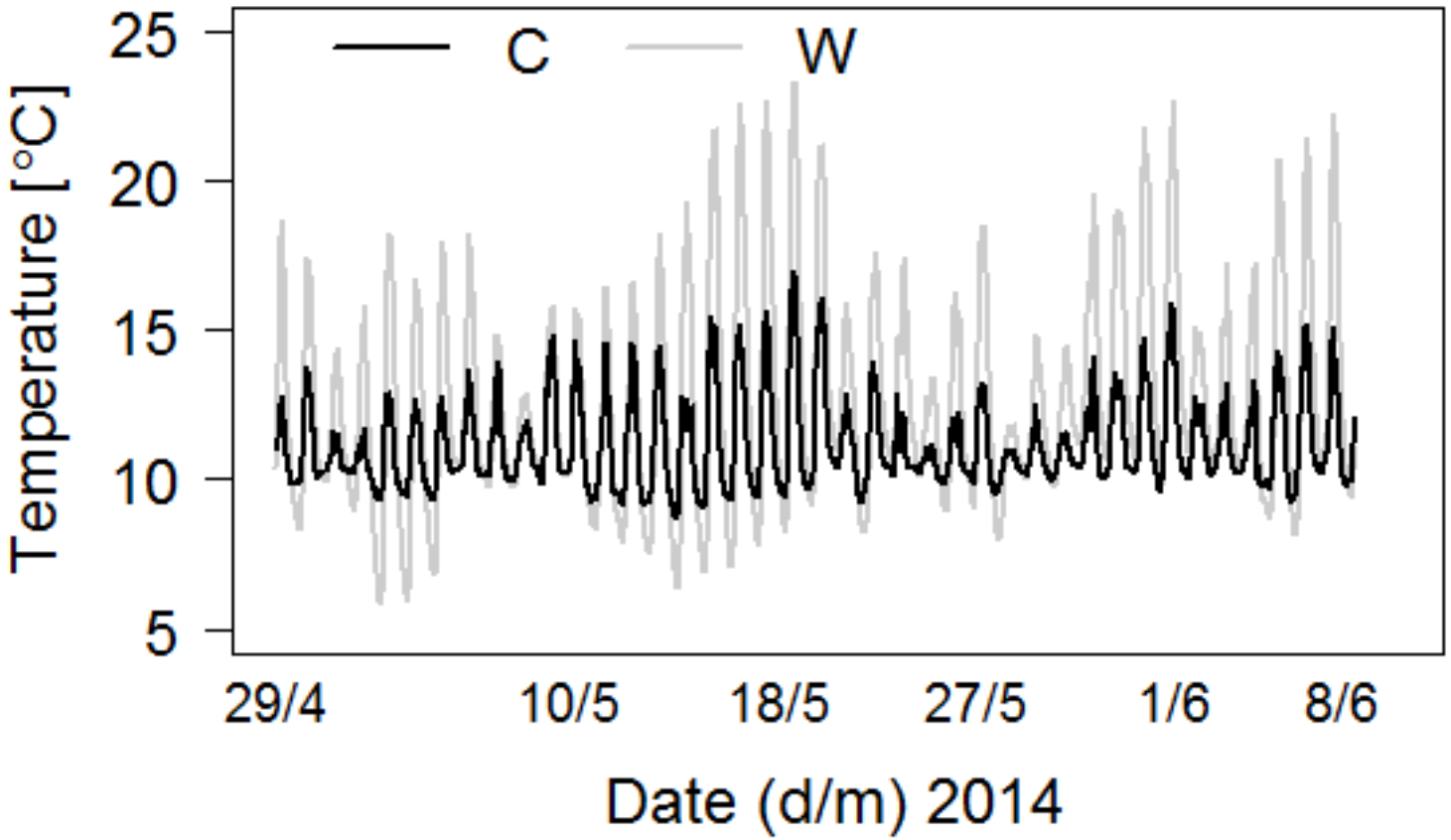
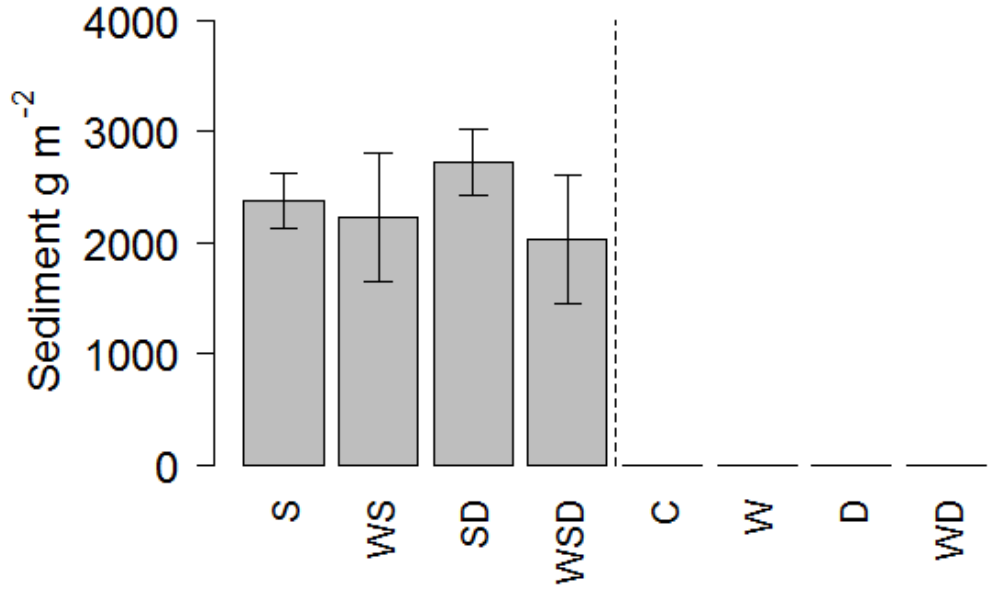


Figure 2.5. **Physical treatment characterisation following stressor application**. Mean (±1SE) sediment mass added to each treatment (a) (vertical dashed line separates treatments by sediment); and mean (±1SE) channel water depth among treatments (b) (vertical dashed line separates treatments by dewatering) where pools refer to deeper central section of channels. Treatment labels denote the following: C = control, S = sediment, D = dewatered, W = warmed.

(a)



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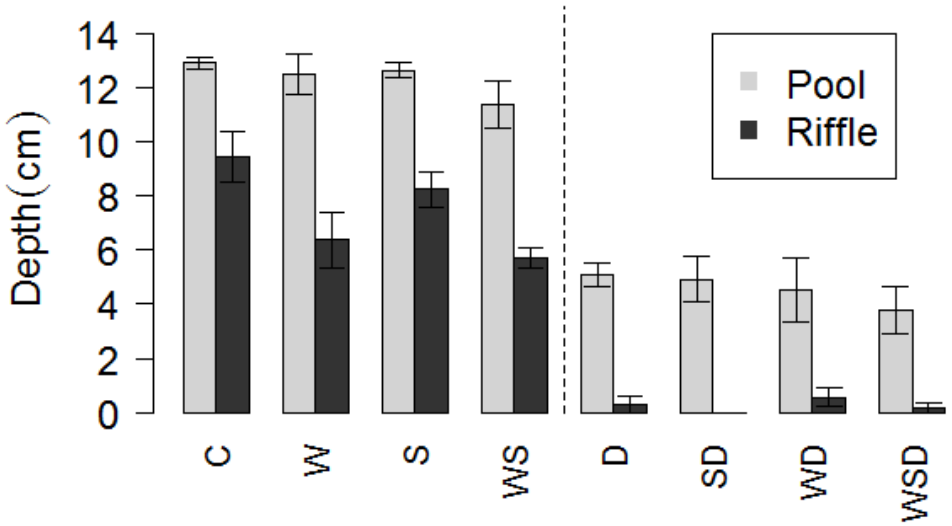
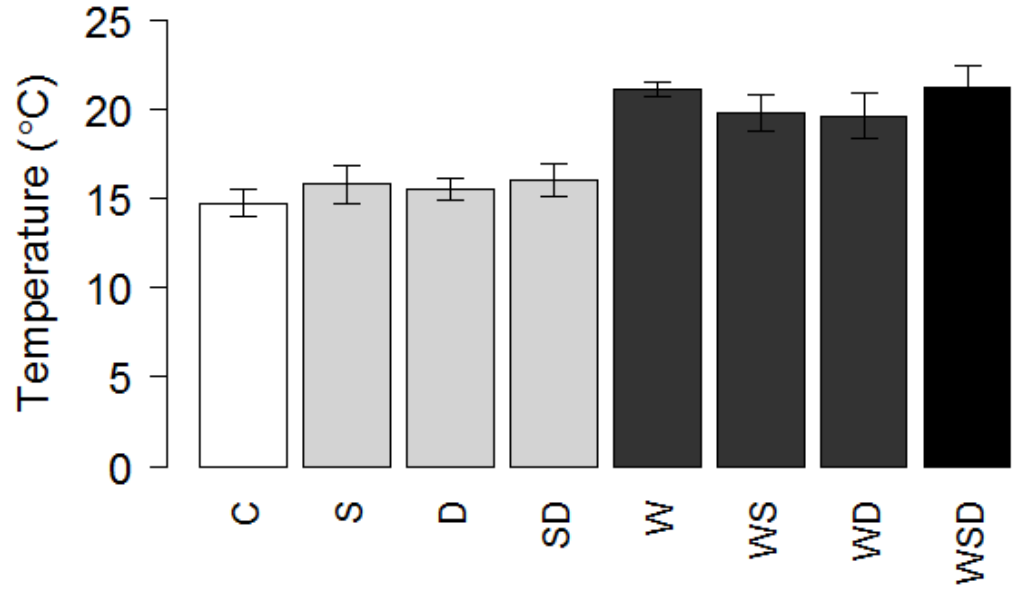


Figure 2.6. **Mesocosm treatment effects on temperature and dissolved oxygen.** Mean water temperature maxima (a) and dissolved oxygen minima (b) during the experiment. Values represent mean values from the four weekly spot readings (usually taken ~midday). Treatment labels denote the following: C = control, S = sediment, D = dewatered, W = warmed. Bars illustrate mean values ±1SE. Bar tone denotes number of stressors applied (white = 0; light grey = 1; dark grey = 2 and black = 3).

(a)



(b)

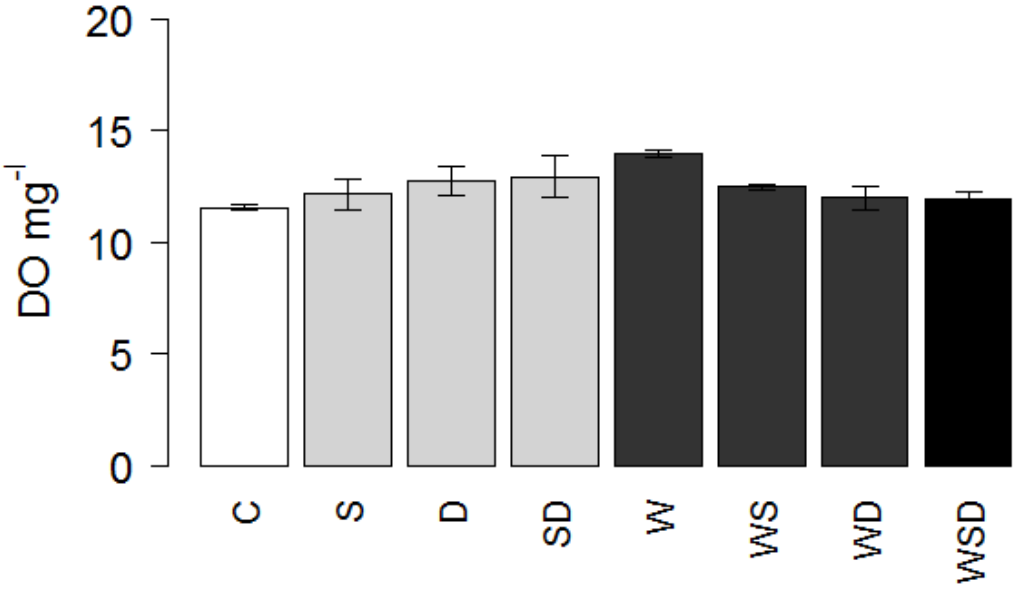
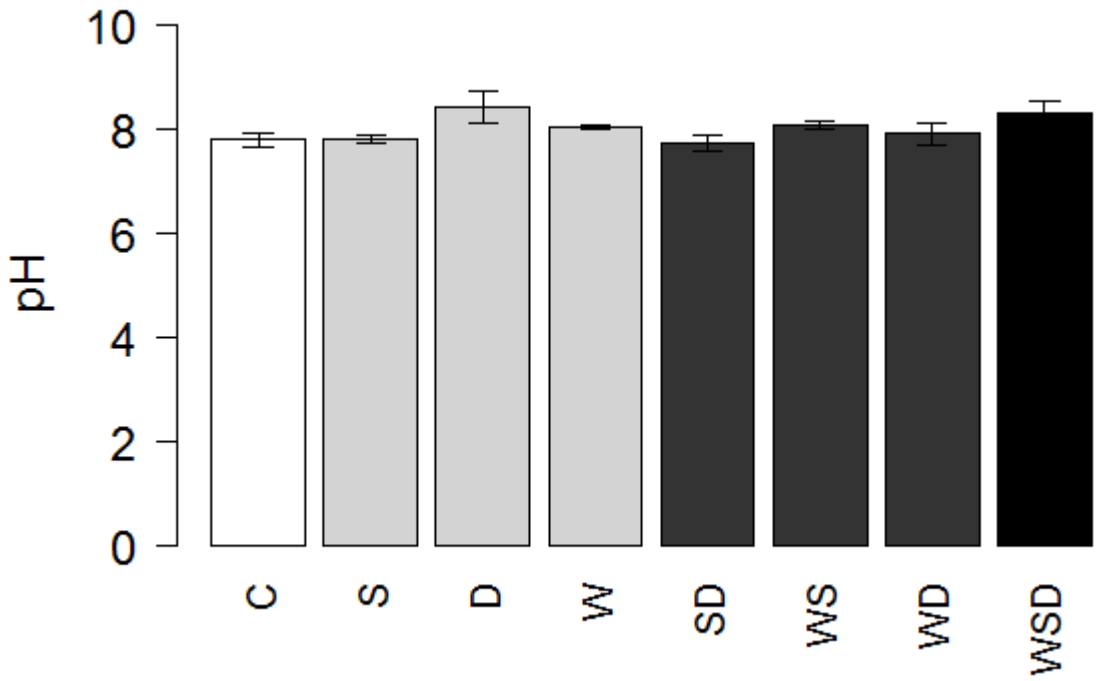


Figure 2.7. **Mesocosm treatment effects on pH.** Recorded at the end of the experiment. Treatment labels denote the following: C = control, S = sediment, D = dewatered, W = warmed. Bars illustrate mean values ±1SE. Bar tone denotes number of stressors applied (white = 0; light grey = 1; dark grey = 2 and black = 3).



2.4.2 Treatment responses

In total, 9610 macroinvertebrate individuals spanning 44 taxa were collected from the channels at the end of the experiment (Table A1, Appendix A). The most abundant taxa were *Micropsectra* sp. (32.7% of individuals); Oligochaeta spp. (19.9%); *Chaetocladius dentiforceps* type (14%); *Gammarus pulex* (9.3%); *Radix balthica* (6.7%); *Helobdella stagnalis* (3.3%); *Caenis luctuosa* (2.5%); *Orthocladius* S type/ *Paratrichcoladius* (2.5%); *Macropelopia* sp. (2.4%); *Synorthocladius semivirens* (1.1%) and *Polycelis nigra* (1.1%).

Three way ANOVA demonstrated that temperature, dewatering and sediment had no statistically significant main effects on species richness or total density (Table 2.4; Fig. 2.8). However, interactions between stressors were significant for temperature x sedimentation (three way ANOVA, P = 0.003; Table 2.4), with warming significantly increasing total density only when combined with sediment (Tukey HSD, P = 0.015). A significant temperature x dewatering (three way ANOVA, P <0.001; Table 2.4) interaction was also revealed, with total density significantly lower when dewatering application was combined with warming (Tukey HSD, P = 0.015). Species richness was not significantly different among treatments (three way ANOVA, P >0.004; Table 2.4).

Partial redundancy analyses revealed a significant effect of all treatments on macroinvertebrate community structure (Table 2.3, analyses 1 and 2) for both square root transformed abundance (axes 1 and 2 explained 37.6% of total variance in species data, P = 0.001, Fig. 2.9) and relative abundance (31.3% variance, P = 0.001, Fig. 2.10). For 20 taxa, > 20% of variance was explained by the ordination model on square root transformed abundances (Fig. 2.9, with mean densities for

selected taxa presented in Figs 2.11-2.16) including *A. fluviatilis*, *Chaetocladius dentiforceps* type, *G. pulex, H. stagnalis*, *Micropsectra* sp., Oligochaeta spp., *Pisidium* sp., *P. nigra* and *R. balthica*. Absolute and relative RDA analyses were similar, but a few taxa vectors differed, e.g. of all *Drusus anulatus* individuals identified, more were associated with WS and WSD treatments, but relative to total taxa densities, were associated more with W and WD. This may be due to greater relative densities of *Micropsectra* sp. in WS and WSD channels. Pairwise partial RDA analyses (Table 2.3, analyses 3-9), were consistent with the three way ANOVA, revealing that stressors had no detectable effects when applied singly ($P > 0.05$) on macroinvertebrate community composition, whereas compound stressors (warming and sediment addition [WS], 12.8% variance, $P = 0.001$; Table 2.3; sediment addition and dewatering application [WD], 10.1% variance, $P = 0.005$; Table 2.3; warming and sediment addition and dewatering application [WSD], 8.4% variance, $P = 0.012$; Table 2.3) differed significantly from the control. Taxa vectors for *Micropsectra* sp., *G. pulex* and *R. balthica* elicited the strongest affinity to treatments, with densities greater in warming and sediment addition (WS), control (C) and warming (W), respectively (Fig. 2.9).

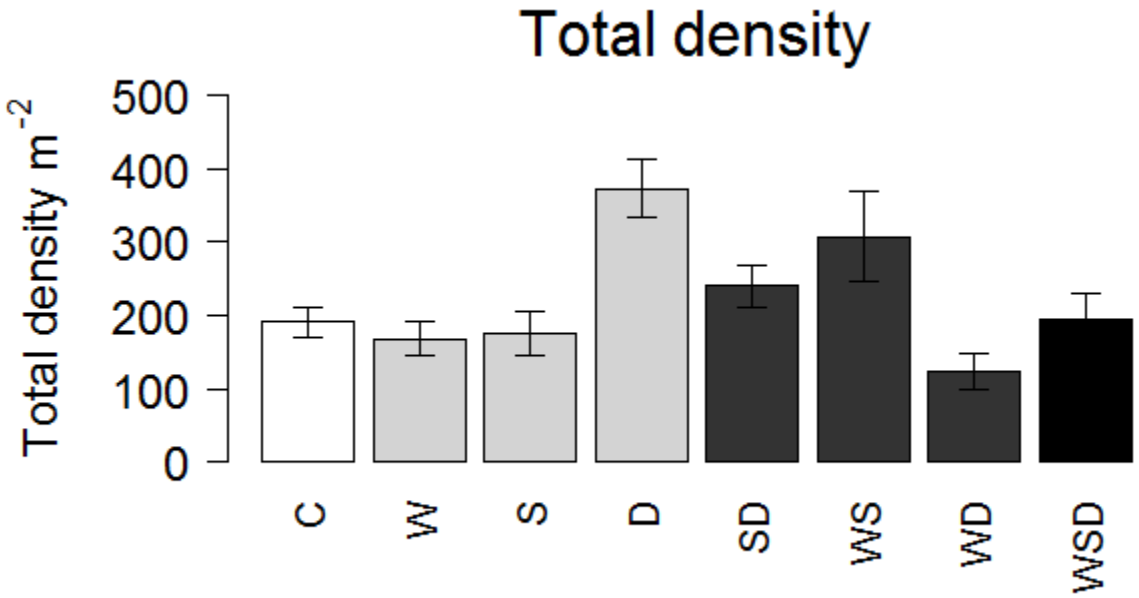
At a population level, significant stressor main effects (n=3) were more prevalent than interaction effects (n=2; Table 2.4, Figs. 2.10-2.16). Warming elicited a significant main effect on *R. balthica* by increasing overall taxa density (three way ANOVA, $P = <0.001$, Fig. 2.15b; Table 2.4). A weak main negative effect of warming on *Chaetocladius dentiforceps* type was observed (Fig. 2.11b) but this was not statistically significant (three way ANOVA, $P = 0.072$; Table 2.4). There was a main effect of sediment on *Micropsectra* sp. (ANOVA, $P = <0.001$, Fig. 2.13b, Table 2.4)

and *P. nigra* (three way ANOVA, *P* = <0.001, Fig. 2.15a; Table 2.4), with densities strongly increasing or declining in sediment addition channels, respectively. Dewatering had no significant main effect on taxa populations (three way ANOVA, *P* >0.05; Table 2.4). *Caenis luctuosa* was significantly affected by an interaction between temperature and dewatering (three way ANOVA, *P* = 0.001, Fig. 2.11a; Table 2.4) with lower densities when warming and dewatering application were combined (WD) relative to warming (W) alone (Tukey HSD, *P* = 0.002). An interaction between temperature and sediment affected densities of *S. semivirens* (three way ANOVA, *P* = <0.001; Table 2.4), where warming (Tukey HSD, *P* = 0.013) and sediment (Tukey HSD, *P* = 0.002) significantly reduced densities relative to control singly, but when combined elicited a neutral response relative to the control.

Biofilm biomass was affected by an interaction between temperature and sedimentation (three way ANOVA, *P* = 0.03; Fig. 2.17), revealing a lower biomass when warming and sediment addition were combined (WS), relative to warming (W) alone.

Figure 2.8. **Community level treatment effect responses.** Values indicate mean (±1SE) total density (a) and taxon richness (b) across treatments. Treatment labels denote the following: C = control, W = warmed, S = sediment, D = dewatered. Bar tone denotes number of stressors applied (white = 0; light grey = 1; dark grey = 2 and black = 3).

(a)



(b)

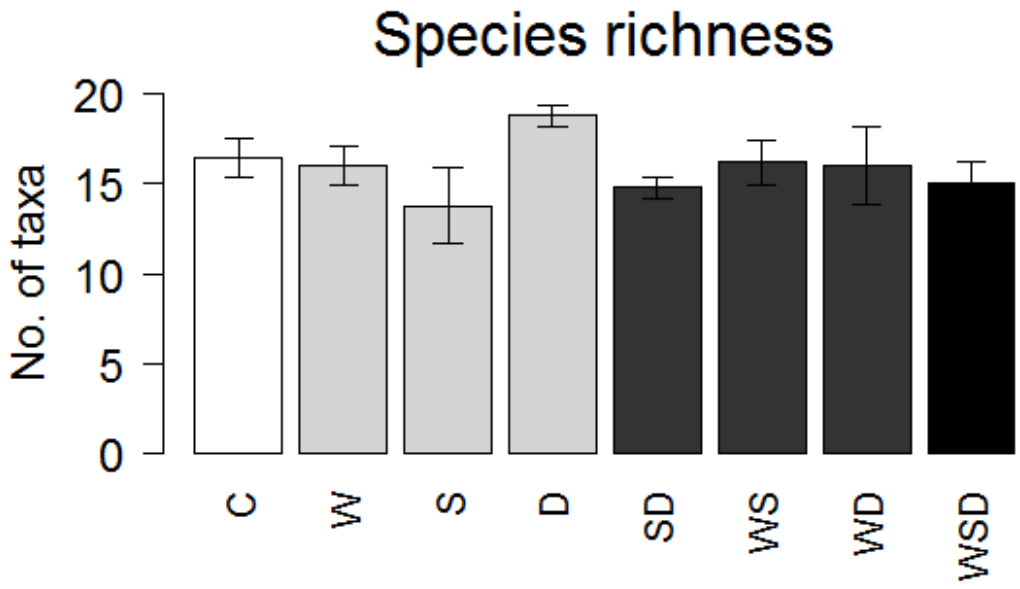


Figure 2.9. **RDA ordination diagram of square root transformed taxa abundance.** Showing differences in macroinvertebrate community structure among treatments (C = control, S = sediment, D = dewatered, W = warmed). Vector direction and length indicates the trend and extent, respectively, of population abundance among treatments. Taxa are abbreviated at the end of vectors: Anc = *Ancylus fluviatilis*; Cae = *Caenis luctuosa*; Cer = *Ceratopogoninae* sp.; Cha = *Chaetocladius dentiforceps*; Dru = *Drusus anulatus*; Gam = *Gammarus pulex*; Hel = *Helobdella stagnalis*; Met = *Metriocnemus eurynotus* type; Mic = *Micropsectra* sp.; Oli = Oligochaeta spp.; Pis = *Pisidium* sp.; Pro = *Prodiamesa* sp.; Rad = *Radix balthica* and Syn = *Synorthocladius semivirens*.

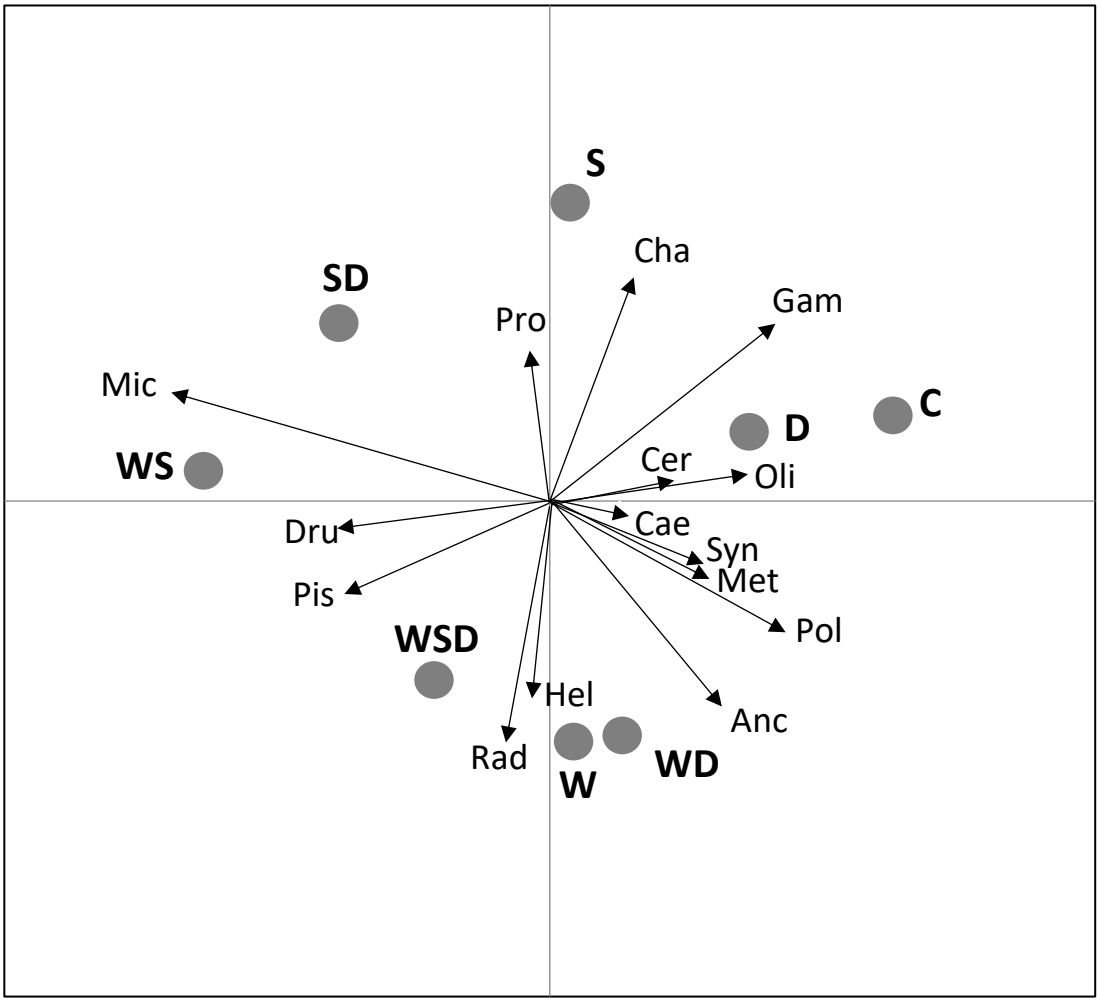


Figure 2.10. **RDA ordination diagrams of relative taxa abundance.** Showing differences in macroinvertebrate community structure among treatments (C = control, S = sediment, D = dewatered, W = warmed). Vector direction and length indicates the trend and extent, respectively, of population abundance among treatments. Taxa are abbreviated at the end of vectors: Anc = *Ancylus fluviatilis*; Ase = Asselus aquaticus; Cae = *Caenis luctuosa*; Cha = *Chaetocladius dentiforceps*; Dru = *Drusus anulatus*; Gam = *Gammarus pulex*; Hel = *Helobdella stagnalis*; Mac = *Macropelopia* sp.; Met = *Metriocnemus eurynotus* type; Mic = *Micropsectra* sp.; Oli = Oligochaeta spp.; Pis = *Pisidium* sp.; Pla = *Planorbis planorbis*; Pol = *Polycelis nigra*; Rad = *Radix balthica* and Syn = *Synorthocladius semivirens*.

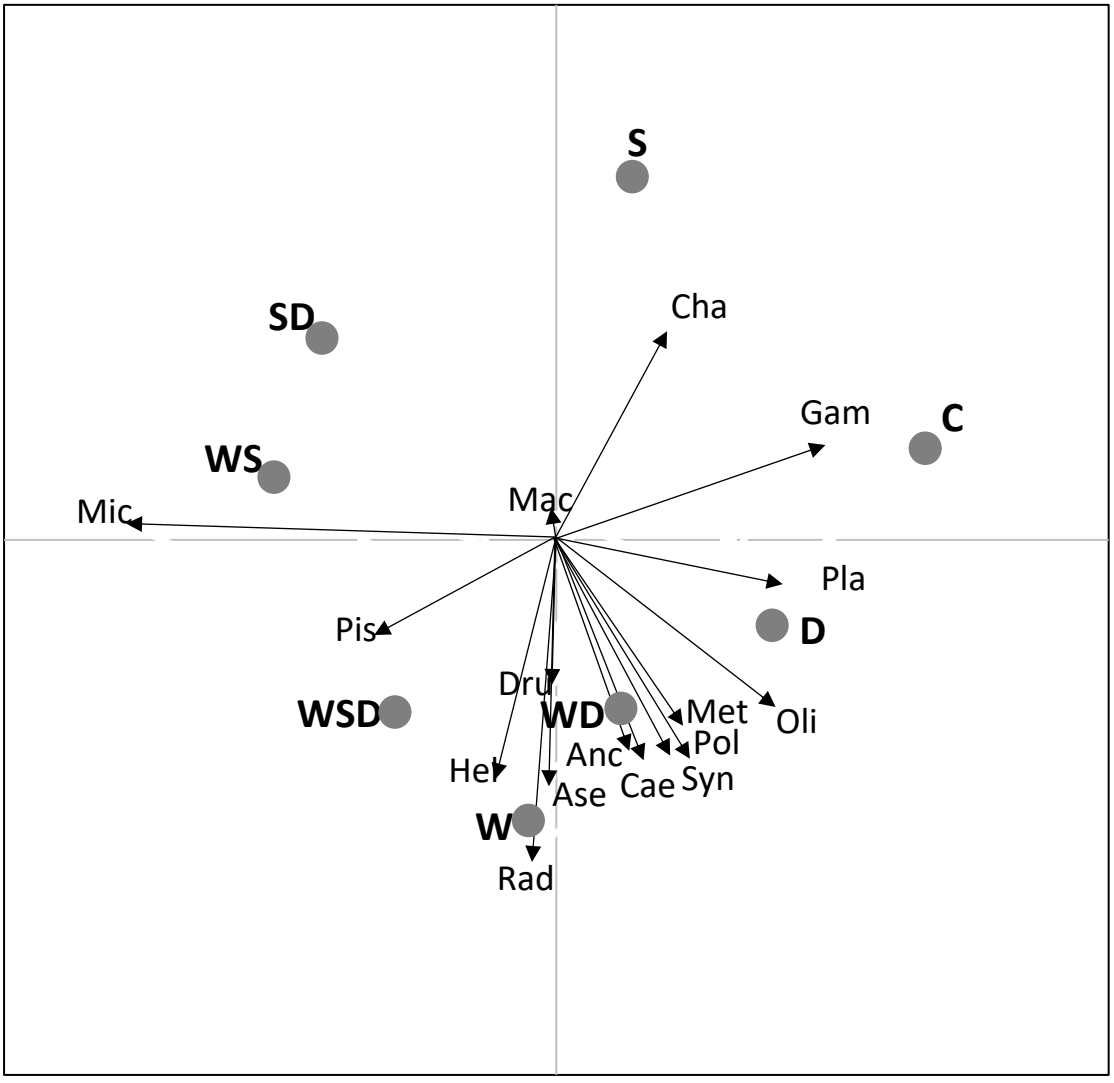
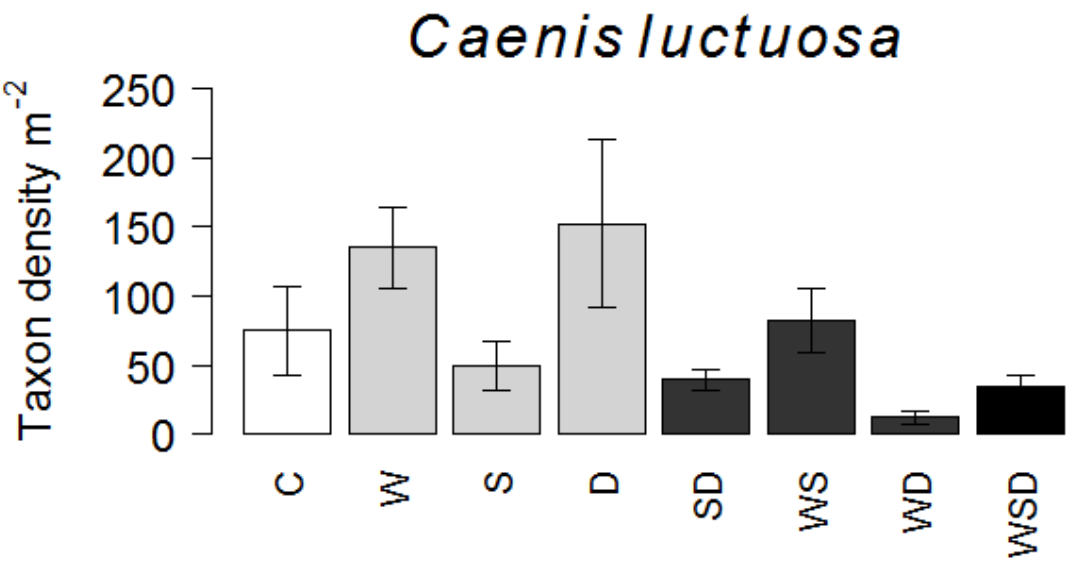


Table 2.3. **Redundancy analysis model summary table*.** Table shows effect of treatments on macroinvertebrate community structure. Constrained (analysis 1-2) and partially constrained (3-9) RDA statistics. Significant *P* values are shown in bold. Treatment labels denote the following: C = control, S = sediment, D = dewatered, W = warmed. Explanatory variables and covariables indicated by codes: C = control, S = sediment, W = warming and D = dewatering.

Redundancy analysis	Explanatory variables	Covariables	% var	Trace	*F*	*P*
1	C, S, D, W, WS, WD, SD, WSD	0 passive.	37.6	0.313	2.078	**0.001**
2	C, S, D, W, WS, WD, SD, WSD	0 passive.	31.3	0.376	2.751	**0.001**
3	C, W	S, D, WS, WD, SD, WSD	6.0	0.044	2.057	0.059
4	C, S	D, W, WS, WD, SD, WSD	5.6	0.041	1.915	0.094
5	C, D	S, W, WS, WD, SD, WSD	2.6	0.018	0.861	0.516
6	C, WS	S, D, W, WD, SD, WSD	12.8	0.101	4.708	**0.001**
7	C, WD	S, D, W, WS, SD, WSD	5.9	0.043	2.011	0.065
8	C, SD	S, D, W, WS, WD, WSD	10.1	0.077	3.595	**0.005**
9	C, WSD	S, D, W, WS, WD, SD	8.4	0.063	2.949	**0.012**

Figure 2.11. **Mean (±1SE) density of 12 core taxa in treatments**, showing a) *C. luctuosa* and b) *Chaetocladius* type, where C = control, W = warmed, S = sediment, D = dewatered. Bar tone denotes number of stressors applied (white = 0; light grey = 1; dark grey = 2 and black = 3).

(a)



(b)

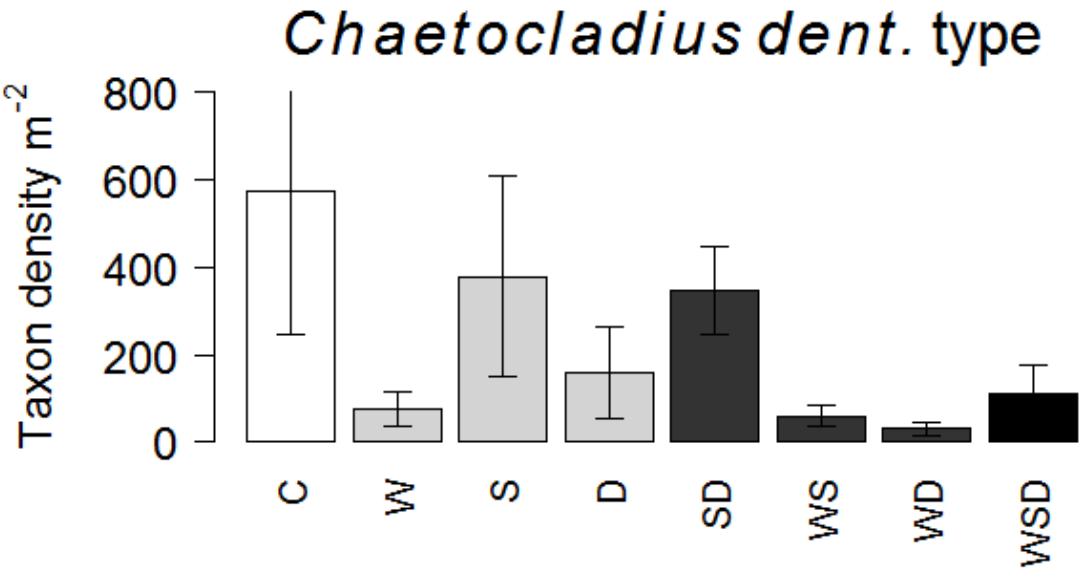
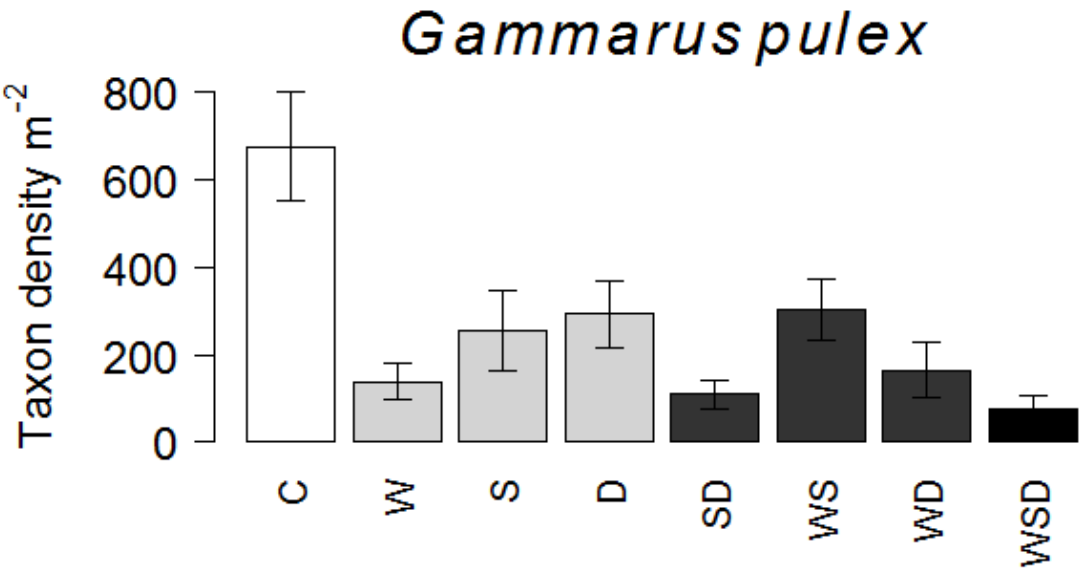


Figure 2.12. **Mean (±1SE) density of 12 core taxa in treatments**, showing a) *G. pulex* and b) *H. stagnalis*, where C = control, W = warmed, S = sediment, D = dewatered. Bar tone denotes number of stressors applied (white = 0; light grey = 1; dark grey = 2 and black = 3).

(a)



(b)

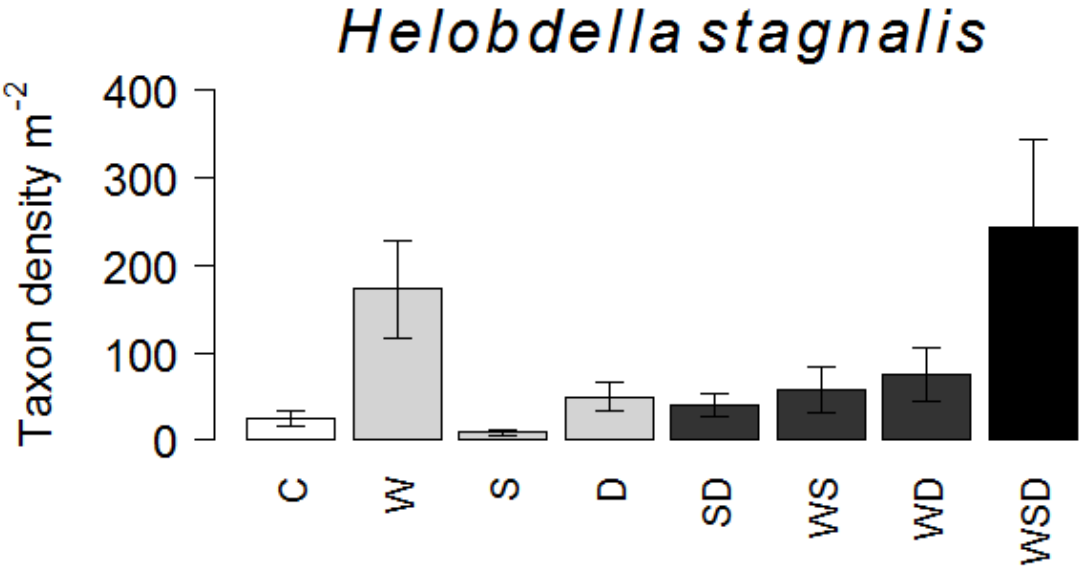
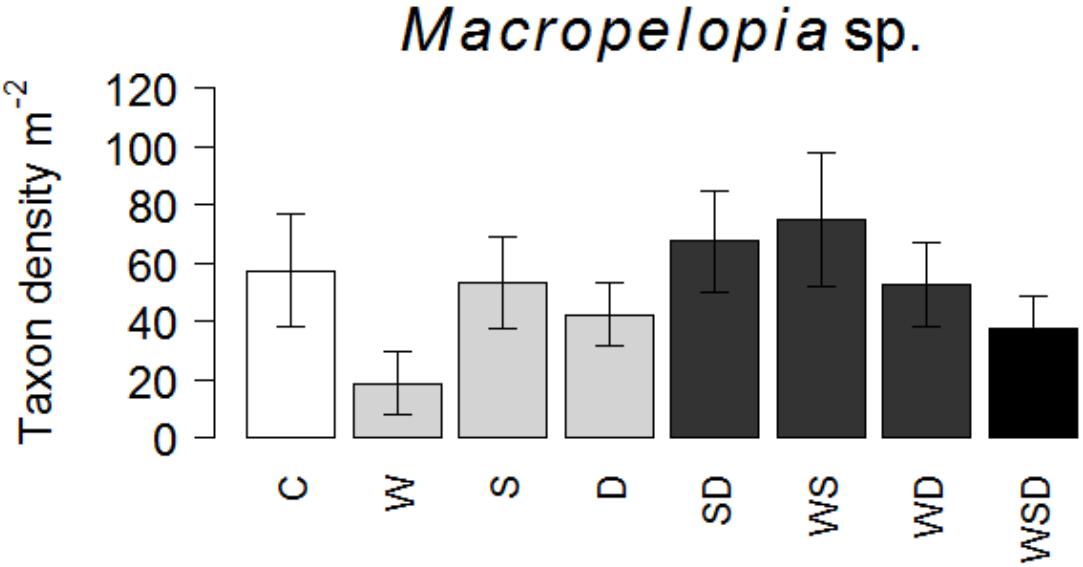


Figure 2.13. **Mean (±1SE) density of 12 core taxa in treatments**, showing a) *Macropelopia* sp. and b) *Micropsectra* sp., where C = control, W = warmed, S = sediment, D = dewatered. Bar tone denotes number of stressors applied (white = 0; light grey = 1; dark grey = 2 and black = 3).

(a)



(b)

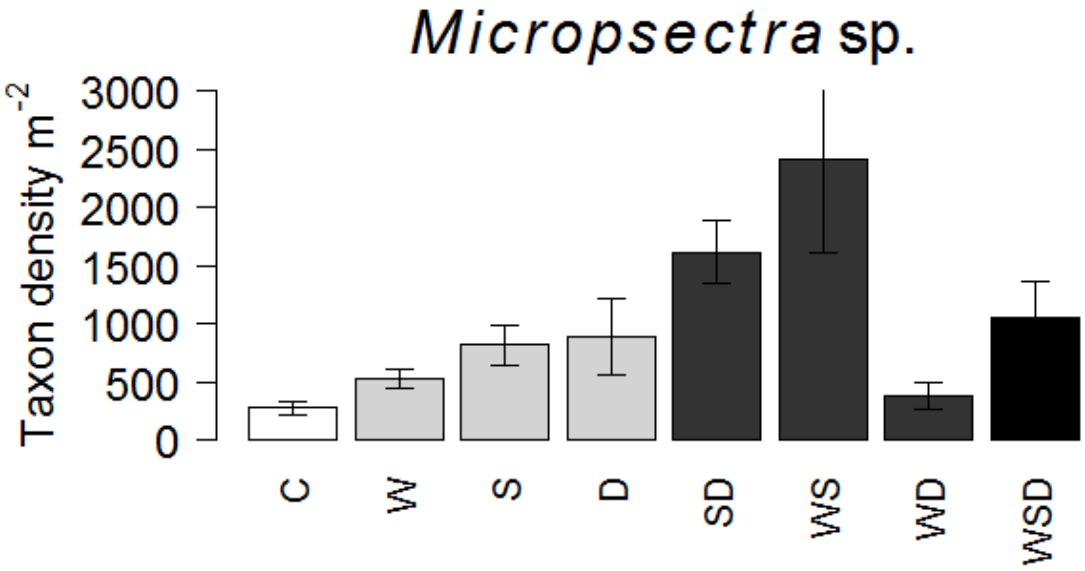
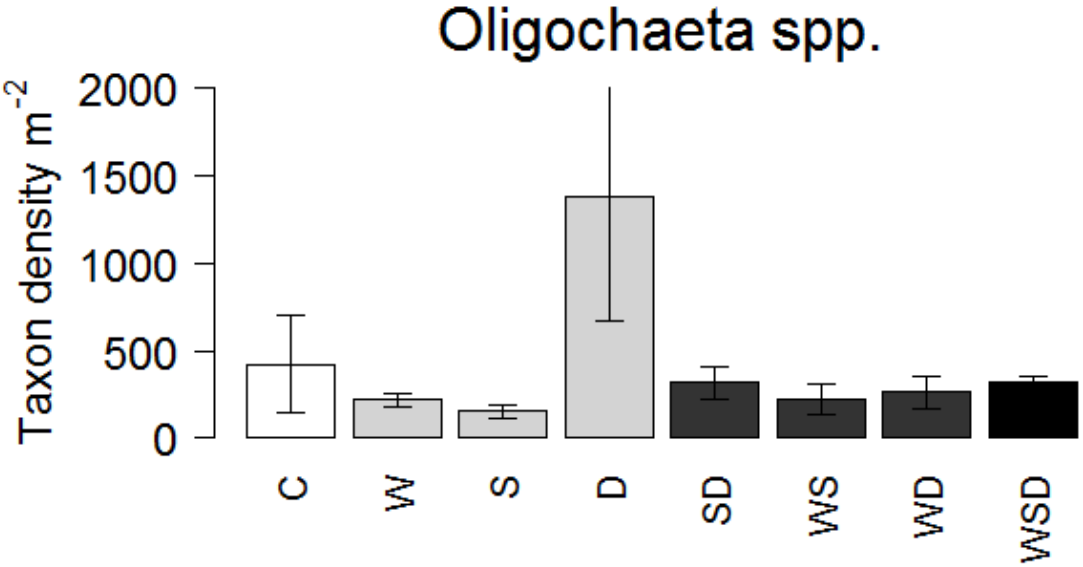


Figure 2.14. **Mean (±1SE) density of 12 core taxa in treatments**, showing a) Oligochaeta spp. and b) *Orthocladius* S-type, where C = control, W = warmed, S = sediment, D = dewatered. Bar tone denotes number of stressors applied (white = 0; light grey = 1; dark grey = 2 and black = 3).

(a)



(b)

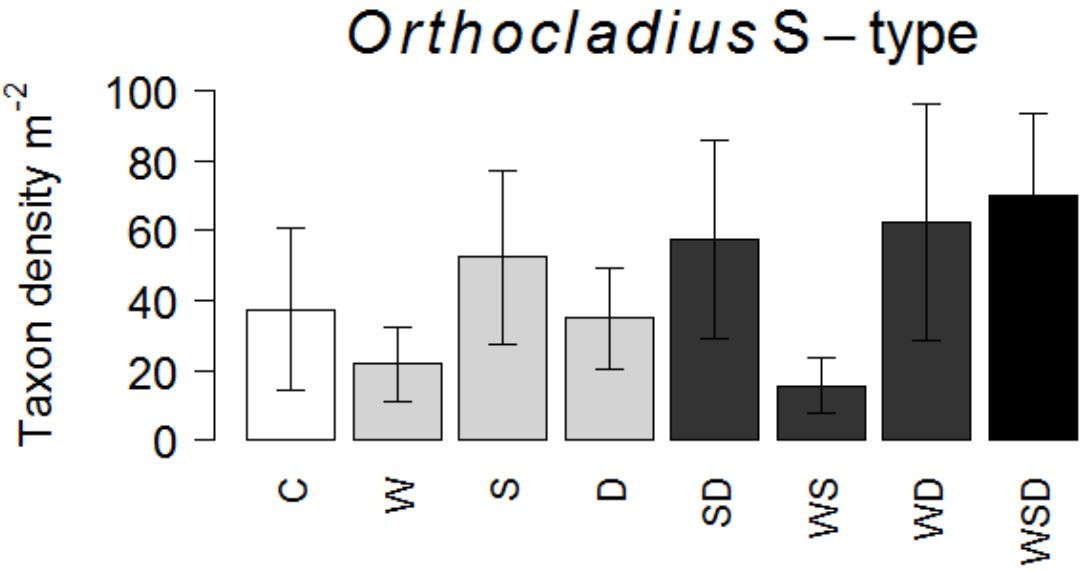
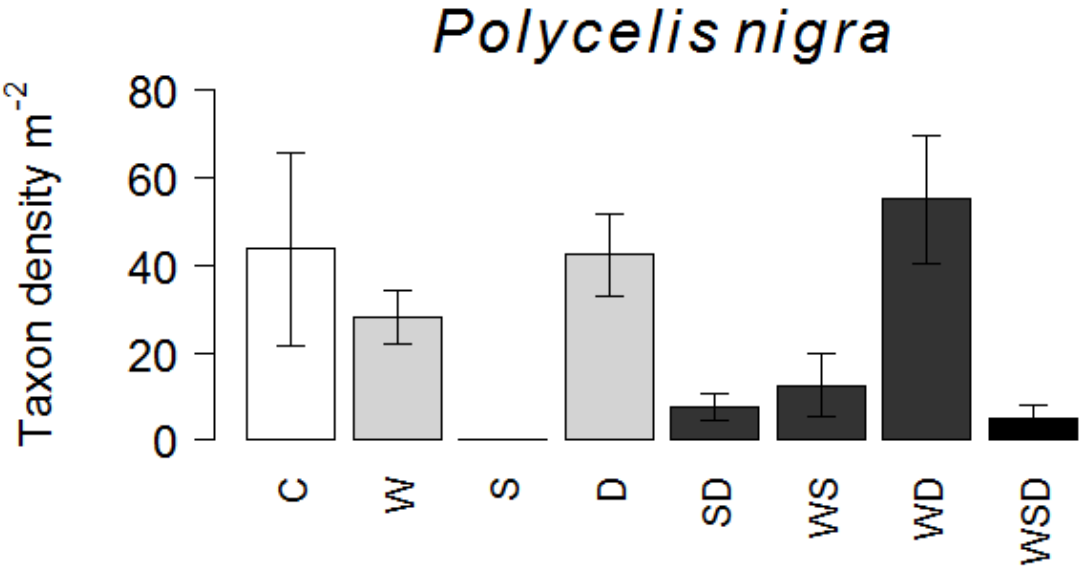


Figure 2.15. **Mean (±1SE) density of 12 core taxa in treatments**, showing a) *P. nigra* and b) *R. balthica*, where C = control, W = warmed, S = sediment, D = dewatered. Bar tone denotes number of stressors applied (white = 0; light grey = 1; dark grey = 2 and black = 3).

(a)



(b)

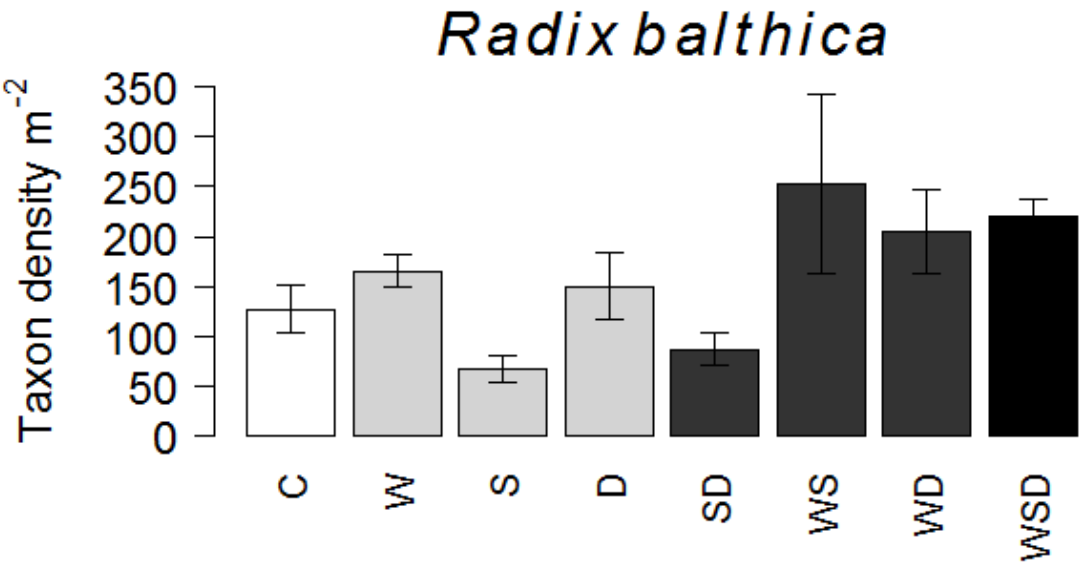
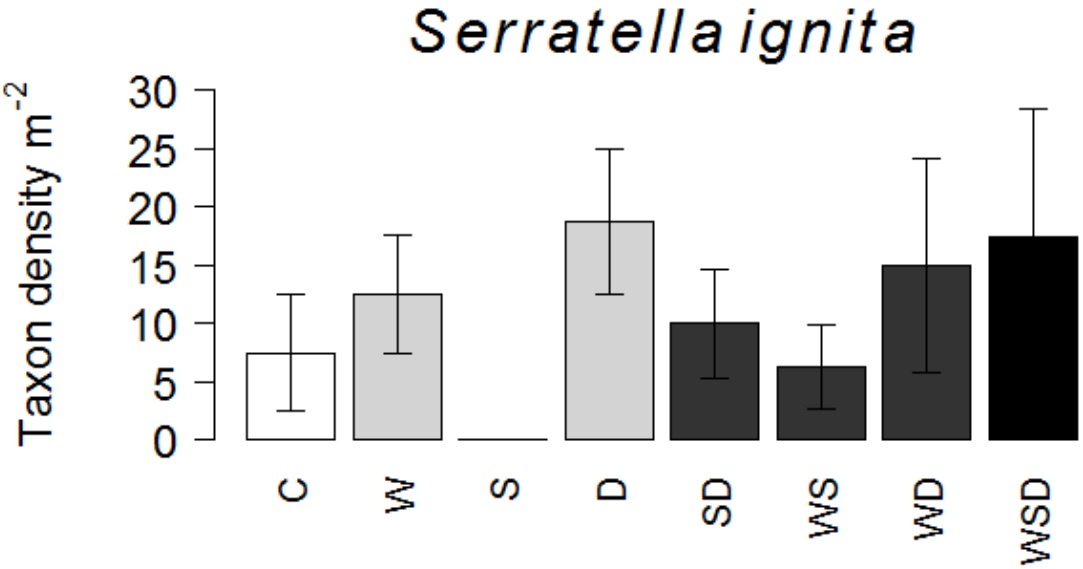


Figure 2.16. **Mean (±1SE) density of 12 core taxa in treatments**, showing a) *S. ignita* and b) *S. semivirens*, where C = control, W = warmed, S = sediment, D = dewatered. Bar tone denotes number of stressors applied (white = 0; light grey = 1; dark grey = 2 and black = 3).

(a)



(b)

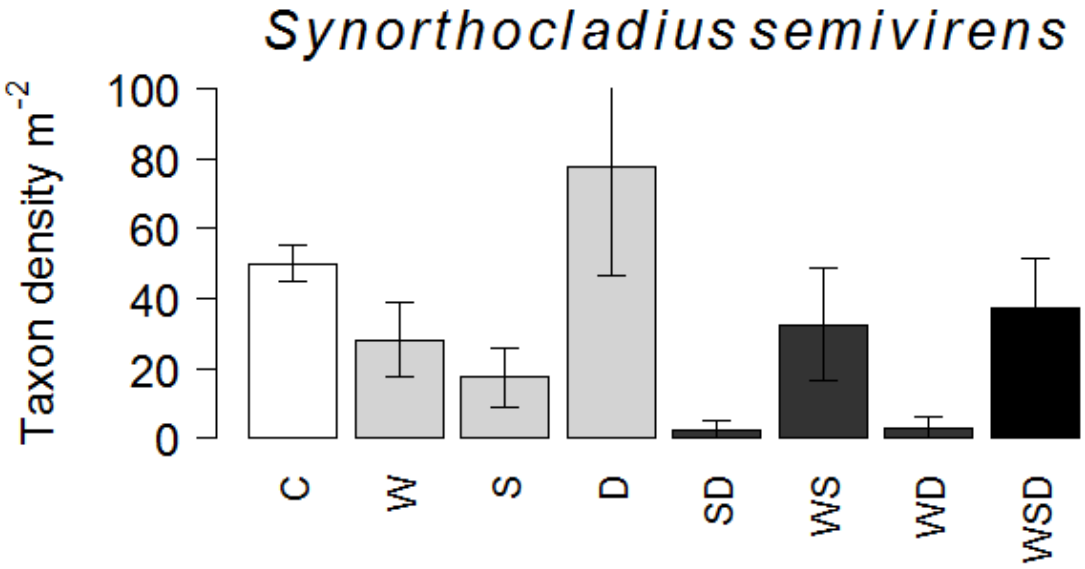
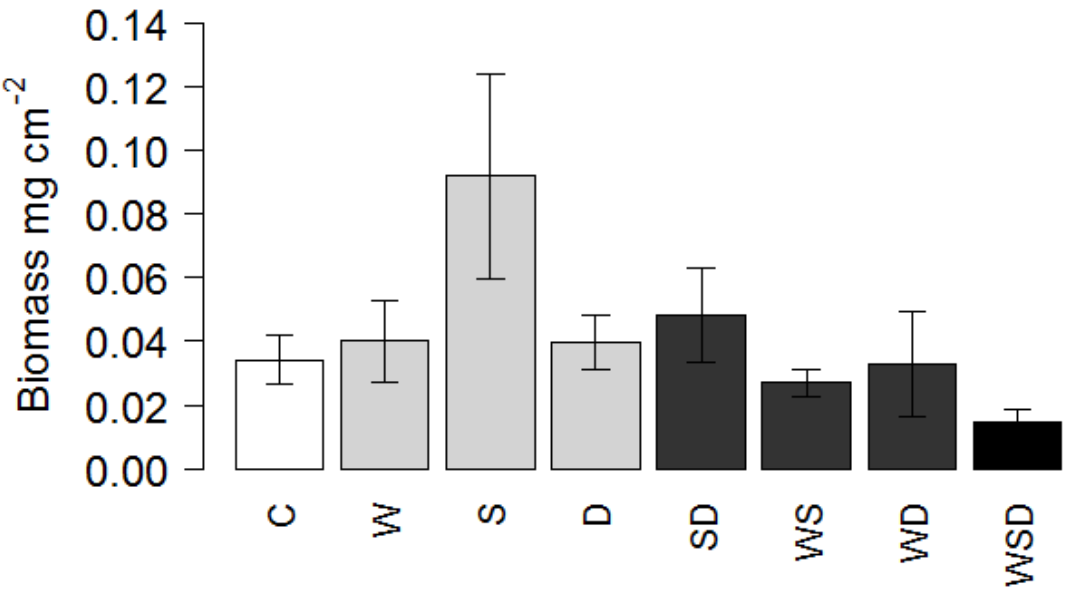


Table 2.4. **Three way ANOVA summary results***. Values show *P* value responses. Significant values (*P* <0.004) shown in bold. 'Temperature', 'sediment' and 'dewatering' show main effects. Other columns indicate interaction effects. Bottom row illustrates total number of significant treatment effects.

Dependent variable	Temperature	Sediment	Dewatering	Temperature x sediment	Temperature x dewatering	Sediment x dewatering	Warming x sediment x dewatering
Species richness	0.878	0.065	0.574	0.144	0.244	0.507	0.959
Total density	0.036	0.183	0.988	**0.003**	**<0.001**	0.421	0.548
Caenis luctuosa	0.447	0.453	0.058	0.162	**0.001**	0.364	0.019
Chaetocladius den type	0.072	0.607	0.833	0.853	0.513	0.481	0.621
Gammarus pulex	0.022	0.140	0.008	0.043	0.847	0.327	0.137
Helobdella stagnalis	0.078	0.375	0.110	0.712	0.617	0.176	0.735
Macropelopia sp.	0.364	0.147	0.566	0.694	0.959	0.070	0.056
Micropsectra sp.	0.436	**<0.001**	0.999	0.876	0.005	0.830	0.615
Oligochaeta spp.	0.288	0.309	0.129	0.154	0.374	0.810	0.245
Orthocladius S type	0.877	0.261	0.412	0.904	0.467	0.794	0.159
Polycelis nigra	0.622	**<0.001**	0.326	0.667	0.144	0.983	0.458
Radix balthica	**<0.001**	0.219	0.337	0.028	0.770	0.842	0.835
Serratella ignita	0.610	0.147	0.118	0.592	0.106	0.796	0.798
Syn. semivirens	0.664	0.090	0.167	**<0.001**	0.972	0.725	0.050
TOTAL:	1	2	0	2	2	0	0

*see Table A2, Appendix A for three way ANOVA model outputs

Figure 2.17. **Mean (±1SE) biofilm biomass among treatments**. Treatment labels denote the following: C = control, W = warmed, S = sediment, D = dewatered. Bar tone denotes number of stressors applied (white = 0; light grey = 1; dark grey = 2 and black = 3).



2.5 DISCUSSION

Extreme events such as drought are predicted to increase in frequency and intensity in future (IPCC, 2013; Vidal & Wade, 2009; Blenkinsop & Fowler, 2007), and the impetus to understand the ecological effects of such events is now greater than ever (Ledger & Milner, 2015). Despite growing knowledge of community structure responses to drought, a research gap regarding underpinning mechanisms of drought responses has been identified. This chapter provides some of the first results exploring the multiple stressors of drought, and in particular illustrated that compound drought stress is more important than independent stressor effects on macroinvertebrate community structure (but main effects affected populations, both increasing and reducing densities of particular taxa). Moreover, interactions among stressors were found to be more common than stressor main effects (the latter where main effect size is greater than higher level interaction effects size, see Piggott *et al*., 2015) highlighting the complexity of compound disturbances in determining community structure.

As predicted in Hypothesis 1, sediment was the most pervasive stressor, responsible for 67% of significant ANOVA main effects on population densities. However, the direction of impact varied, with positive effects for *Micropsectra* sp. (i.e. greater abundance) and negative effects for *P. nigra* (lower abundance), supporting the prediction that the direction of taxon responses will vary as made in Hypothesis 6. Species-specific responses to drought have also been found by Lancaster & Ledger (2015) reflecting the varying sensitivity of taxa to stress (Dewson *et al*., 2007). Pairwise RDA models illustrate that all compound sediment treatments (i.e. WS, WSD, SD) significantly explained community structure

variance, supporting predictions that combined stressor treatments will have a greater impact than single stressor treatments, as made in Hypothesis 7. Inspection of ordination plots demonstrate that most taxon vectors were orientated away from all sediment treatments (i.e. main effect), illustrating overall deleterious sediment impacts and supporting Hypotheses 1 and 2. Despite sediment main effects at the population level, community level effects (species richness, total abundance) were not detected. It is believed a counterbalance between directional impacts on taxa populations resulted in no overall effect on total density being identified (Piggott *et al*., 2015). Sediment interacted with temperature for 50% of identified significant interactions, resulting in community (richness and total density) and population level effects. At the population level, the negative effect of warming and sediment density on *S. semivirens* was lost when the two stressors were combined, resulting in a mean density in the compound treatment similar to the control (i.e. neutral effect). On the other hand, at the community level, the effect of warming on total density was dependent on sediment (i.e. facilitation of stressors), with total density in warmed (W) channels only significantly greater than the control mean when combined with sediment (WS) (due to greater representation by *Micropsectra* sp.) supporting Hypothesis 7. *Chaetocladius dentiforceps* type displayed a strong affinity towards sediment (S), suggesting possible utilisation of significantly elevated biofilm biomass for resources and / or habitat. Negative main effects of sediment on specific taxa (e.g. *P. nigra*) suggests possible smothering effects by fine sediment on this slow moving taxon (Chandrasekara & Frid, 1998; Wood *et al*., 2005) and / or that fine sediment decreased the likelihood of taxa remaining within the mesocosms (Jones *et al*., 2015; Piggott *et al*., 2015). Sediment can reduce total invertebrate density (Ciesielka & Bailey, 2001) but it can also increase the density of sediment

tolerant taxa, e.g. Chironomidae spp. (Nuttall & Bielby, 1973; Kochersberger *et al*., 2012). Some Chironomidae spp. (chiefly *Micropsectra* sp.) were responsible for maintaining total density when sediment was applied. These taxa may have preferentially selected sediment treatments in order to utilise the fine particles for case construction (Wood & Armitage, 1997) and elevated POM utilisation. Whilst sediment appeared to have some beneficial taxa effects, it mainly triggered negative responses, suggesting the benefits gained from the quantity applied to the mesocosms (i.e. habitat, resources and nutrients) were outweighed by negative effects such as smothering and clogging of interstitial spaces (Wagenhoff *et al*., 2012).

Warming resulted in a main effect on *R. balthica* density, with densities greater among warmed treatments than unwarmed treatments, contrary to predictions in Hypothesis 3. Furthermore, warming elicited two of the greatest vector lengths in the ordination plots (*R. balthica* and *Micropsectra* sp.), which were strongly orientated towards warmed treatments. Findings from this chapter would therefore suggest that the extent of subjected warming in this experiment had greater positive effects on taxa than negative effects. Quantification of biofilm AFDM among treatments demonstrates how basal allochthonous resources did not significantly differ and thus did not explain increased *R. balthica* density with warming. Another possible mechanism includes elevated hatching success (Pritchard *et al*., 1996) of snail eggs added to channels during seeding, and the fact that *R. balthica* are pulmonate and therefore less affected by possible oxygen supply limitations in the warmer channels. *Radix balthica* elsewhere have shown a preference for warmer water temperatures (Friberg *et al*., 2009; Woodward *et al*., 2010) supporting findings

from this chapter. However negative effects of drought on this species have also been reported (Ledger *et al*., 2012) suggesting context dependant mechanisms may determine the fate of this species. In an agricultural study by Piggott *et al*. (2012), *Micropsectra* sp. also responded positively to sediment, but responded negatively when combined with warming. Whilst no multiple stressor studies have investigated extreme diel temperatures (as opposed to static warming), it is difficult to compare temperature effects in this chapter to other studies. Temperature effects in this chapter were present in 100% of significant interaction effects (compared to 50% for sediment effects and 50% for dewatering effects). Pairwise interactions between temperature and additional stressors have also been shown to be common elsewhere (Piggott *et al*., 2012). In addition to the two sediment interactions outlined in the above paragraph, temperature also interacted with dewatering in two instances to determine the direction of dewatering effects: Dewatering resulted in a significantly greater total density than the control at ambient temperature, but significantly lower total density than the control when coupled with warming, suggesting amplification of stress. At the population level, warming and dewatering were not significantly different from the control when applied singly, but when combined resulted in a significantly lower *Caenis luctuosa* density relative to the control. Thus, the effect of warming from these two examples supports Hypothesis 7, i.e. greater effects when stressors are combined relative to independent stressor effects.

Dewatering elicited no significant main effects, but interacted with warming as described in the above paragraph to elicit two interaction effects. Dewatering had no significant differences on taxon richness, opposing predictions made in

Hypothesis 4 (that dewatering would reduce species richness). However, *post-hoc* testing of the interaction between temperature and dewatering revealed that under ambient temperatures, dewatering resulted in a significantly greater total density in comparison to the control mean (largely due to greater representation by Oligochaeta spp., and *S. semivirens*) in agreement with predictions made in Hypothesis 5 – that dewatering would increase population densities within confined space. Similar findings have been found elsewhere (Covich *et al*., 1999; Dewson *et al*., 2007; Wright & Berrie, 1987) suggesting that the aggregation of taxa may be a common response during the initial stages of drought. RDA revealed that taxon vectors displayed a weak attraction towards dewatering, although Oligochaeta spp. vector demonstrated a particularly strong affinity. The interaction between temperature and dewatering for *C. luctuosa* density, and total macroinvertebrate density (resulting in significantly lower density means when both stressors combined), may indicate non-additive effects of combined stressors on macroinvertebrate upper thermal tolerances (Pandolfo *et al*., 2010; Porter *et al*., 1999). Whilst water temperature maxima were insufficiently warm to extirpate taxa in this experiment (Chapter 5), the added stress of dewatering is believed to have either lowered the physiological threshold of particular taxa, or simply led to unfavourable conditions prompting drift. As the lethal / sub-lethal effects cannot be separated here, further work is needed to determine the exact causal mechanisms underpinning these taxa responses to warming.

Gammarus pulex was the only taxon to display strong sensitivity to all three stressors. *G. pulex* vectors demonstrated high affinity to the control (C) in ordination bi-plots, and reduced mean abundance across all treatments, relative to the control.

Whilst these trends were not considered significant due to the large number of ANOVA models ran (i.e. made insignificant by Bonferroni correction), these data suggest that this key species may be particularly sensitive to drought stress, which could have implications upon important stream processes (see Chapter three). No interactions between stressors in this chapter support the community stress-induced hypothesis whereby the sensitivity to a stressor is strengthened when combined with an additional stressor (i.e. additive effect) (Vinebrooke *et al.*, 2004). Instead, interactions were found to be of two kinds: the effect of a stressor is dependent on the effect of another (i.e. facilitation; mostly all interactions were these) or the combined effect of two stressors reduced the sensitivity of both (i.e. inhibition), resulting in a neutral effect (e.g. *S. semivirens*).

Altered community structure was always due to changes to taxon abundances, and never due to taxon richness, or composition. Such effects mirror findings from Woodward *et al*. (2015), Hille *et al*. (2014) and Dewson *et al*. (2007) whereby population densities responded significantly to disturbance when all other community composition metrics including richness remained unperturbed. These findings suggest that the results from this chapter may be a common response to drought. Contrasting directions of species-specific responses in this study cancelled each other's effect resulting in little to no overall change at the level of the community (Mustonen *et al*., 2016), i.e. total abundance. This demonstrates how disturbances such as drought lead to clear winners and losers – e.g. densities of taxa sensitive to sediment such as *P. nigra* declined whilst *r*-selected taxa such as *Micropsectra* sp. gained in abundance. This work would benefit from further investigation of drought winners and losers to determine if shifts in community

composition can continue to support energy flows in food webs and maintain functional processes.

The findings from this study also suggest community resistance among taxa within the mesocosm channels; owing to no differences in species richness between the control and stressed treatments. Whilst resistance may be expected within temporary streams (Bogan *et al.*, 2015), species richness is generally believed to decline in perennial systems in response to drought disturbance events (Lake, 2003; Dewson *et al*., 2007), though this latter study investigated total dewatering. Furthermore, species richness decline to drought has been demonstrated within a drought mesocosm experiment (26% reduction; Ledger *et al*., 2012). Numerous rheophilic taxa such as *Rhyacophila* spp. (*septentrionis, dorsalis*), *Baetis* spp. (*buceratus, rhodani*), *Hydropsyche* spp. (*siltalai, pellucidula*), *Heptagenia sulphurea, Polycentropus flavomaculatus* and *Ephemera danica* were observed within the Candover Brook during macroinvertebrate sourcing (Table A3, Appendix A), yet were observed to be extirpated among all treatments shortly after seeding. These observations demonstrate an overall mesocosm response of rheophilic taxa to reduced flow across all treatments inclusive of the control. It was therefore apparent that the reduced flow in the mesocosms imposed a degree of stress across all channels irrespective of treatment type, relative to the source stream, and that the stress imposed in treatments during the experiment was insufficient to entirely eliminate any remaining taxa. These findings support the drought resistance hypothesis, which states that the remnant community following flow cessation are resistant to stress until total desiccation is reached (Boersma *et al*., 2014), whereby a stepped threshold is crossed (Boulton, 1990) and further taxa extirpation occurs.

Moreover the findings from this study suggest that current velocity thresholds may have had greater effects than other stressors on shaping community structure (Hille *et al*., 2014) owing to the loss of species between the source stream and the control treatments being the greatest species richness effect observed during the experiment. An agricultural multiple stressor study incorporating flow reduction by Matthaei *et al*. (2010) found reduced flow to be a key stressor that reduced taxonomic richness and density, highlighting the importance of stagnation even when combined with additional stressors such as sediment.

Altered community composition may also be a common response to drought in running waters, as the community shifts from typical lotic assemblages to typical lentic assemblages (Acuña *et al*., 2005; Stanley *et al*., 1997). However the presence or absence of taxa were highly similar among treatments, further supporting the drought resistance hypothesis (Boersma *et al*., 2014). Altered community composition can also be attributable to a loss of larger bodied taxa due to stress (Bogan & Lytle, 2011; Jellyman *et al*., 2014; McHugh *et al*., 2010; Woodward et al., 2012) and a replacement by generalist *r*-selected taxa. However, top / intermediate predators were either rare or simply not detected frequently by the sampling regime in the mesocosms, and thus typical predator losses mediated by drought and other disturbances, whereby predators become extirpated, reducing richness, shortening food chain length, and resulting in the partial collapse of the food web (Ledger *et al*., 2013; McHugh *et al*., 2010)), were not detected in the current study.

The temporal scale of the experiment was considered too short to encompass population gains from reproduction and immigration for the majority of taxa, and thus caution should be adopted when interpreting density ‘gain’ effects. As the

mesocosms used were fed from an upwelling borehole, immigration from drift was eliminated. Immigration may have only been possible in the following cases: 1. oviposition from terrestrial adults with rapid multivoltine life cycles, e.g. Chironomidae spp., or 2. from taxa such as molluscs or triclads that can freely move over damp surfaces to colonise from the surrounding amphibious habitat. Therefore, without knowledge of initial densities at the beginning of the experiment, apparent gains in taxa populations must be considered carefully. However the robust experimental design adopted allowed differences in macroinvertebrate community structure endpoints (e.g. total density, richness, population densities) between treatments to be made following a set period of exposure to different stressors. This enabled the importance of individual drought stressors and their interactions to be made. Further work however could adopt a before-after-control-impact (BACI) approach to explore quantifiable population losses and gains to particular drought stressors, which would support the work undertaken in this study. Within temporary aquatic habitats such as isolated pools or small mesocosm channels, changes in taxa densities are driven primarily by emigration and mortality (Drummond *et al*., 2015) and this should be kept in mind when interpreting the direction of treatment mean effects.

The small size of the mesocosms used in this study provided an insight into macroinvertebrate community structure responses to drought (though it must be noted that hyporheic refugia was excluded owing to obvious constraints with artificial mesocosm experiments), as may be expected in small isolated pools following longitudinal stream bed fragmentation and desiccation. However, the small size did exacerbate ‘natural variations’ within treatments, owing to increased

localised context dependency of responses (Tonkin *et al*., 2016). For example small differences in habitat at this small scale may have implicated water quality and connectivity (Walters, 2016). This may have thus prevented otherwise significant effects from being included in the interpretation, or even increased the significance of 'chance' effects. Moreover, the constrained size of the mesocosms may exacerbate challenges when upscaling from the experiment to natural stream and river pools (Englund & Cooper, 2003; Glon *et al*., 2016; Weins, 1989). Larger experimental units would not have been appropriate for this study, but the implications associated with small spatial experiments is worthy to note here to aid interpretation of findings.

Management implications

The novel research in this chapter provides an initial understanding of how stream biota at the population and community level respond to multiple drought stressors. Whilst 2/3 stressor main effects elicited positive taxon responses, 2/4 interaction effects resulted in negative effects relative to constituent stressors. Overall, sediment appeared to be particularly deleterious, eliciting a negative main effect upon a triclad predator, and demonstrating overall negative impacts at a community level (ordination models), particularly when combined with additional stressors. These findings build upon existing drought research that, to date, have been largely unable to identify causal mechanisms underpinning observed biotic responses.

Dewatering did not invoke any main effects, nor were interactions between dewatering and sediment detected. Conversely, temperature and sediment main effects were detected, whilst temperature interaction effects comprised 100% of all significant interactions. These findings highlight the importance of additional

stressors other than dewatering, and suggest that whilst water management and conservation efforts in future should focus on retaining sufficient water in the channel during drought to maintain aquatic habitat (by restoring hydromorphology, e.g. incorporation of meanders, stream bed heterogeneity, provision of logs and boulders within the water course, and by reducing groundwater and surface abstractions), so should efforts be made to minimise sedimentation in the run up to drought (e.g. sediment traps, improved catchment land use, riparian buffers, reduced cattle poaching – e.g. gravelling cattle access points, if appropriate) and to reduce extreme water temperature during dewatering events (e.g. enhancing riparian shading). The frequency of significant temperature interaction effects within this chapter is concerning (100% of interactions) as it suggests future stressor interactions during drought may become more frequent when mean temperatures attributable to climate change and temperature maxima attributable to heat waves and hot days are increased. Fortunately, a high propensity of antagonistic interactions throughout this experiment were observed (i.e. in many cases compound disturbances visually appear to have greater densities than would be expected from the sum of single independent stressor effects). Although antagonistic effects do not remove negative effects of stress, they do dampen the effects of combined stressors, resulting in low densities of sensitive taxa persisting during the disturbance rather than being entirely eliminated. Therefore antagonistic interactions may aid stream resilience and recovery following termination of hydrological drought, as opposed to synergistic or even additive effects. Water managers should therefore incorporate multiple stressor interactions into all future decision making processes, as single stressor stand points are no longer sufficient to minimise effects on biota.

2.6 CONCLUSION

When multiple stressors are combined during drought, interaction effects may be more prevalent than main effects. The direction and magnitude of stressor effects in this chapter have been shown to be taxon specific, but further research is needed to determine the importance of context, geographical location and system type on community and population level responses to drought stressors.

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CHAPTER THREE

Drought as a compound disturbance: Part 2

Ecosystem functioning

3.1 ABSTRACT

Coupled climate-hydrology models forecast that the incidence of extreme hydrological events such as hydrologic droughts will increase in future. Drought can be regarded as a compound disturbance that exposes biota to extremes of low flow, high temperature and excess sedimentation. Both the independent and interactive effects of these stressors on ecosystem processes remain poorly understood in streams. Research in this chapter tested the effect of three drought stressors (dewatering, sedimentation and warming) – applied singly and in combination – on a suite of functional attributes of stream ecosystems, specifically: macroinvertebrate biomass standing stock; macrophyte primary production parameters; organic matter decomposition and stream metabolism (GPP, ER, NEP & benthic respiration). Stressors invoked main effects as well as two and three-way interactions, resulting in sometimes highly complex interactions among the levels of all three stressors. Significant effects were detected at all levels of ecological complexity, but links between each ecological level (e.g. between shredder biomass and macroinvertebrate mediated decomposition) were not apparent. Generally sediment was the most deleterious stressor, reducing total and microbial decomposition whilst having potentially positive effects on other receptors e.g. *Berula erecta* photosynthetic capacity. Temperature was also present in numerous detected interactions. This chapter provides some of the first research to identify the importance of specific drought stressors that underpin a broad spectrum of ecosystem functioning processes. It also highlights the necessity for further research to determine mechanisms that link drought stressor responses across multiple levels of ecological complexity.

3.2 INTRODUCTION

Extreme climatic events are expected to increase in future as a result of climate change (IPCC, 2013). It is generally accepted that a future climate will elicit a mean increase in temperature overlain with extremes in climate variability (IPCC, 2012). Altered rainfall patterns could modify the hydrological regimes of streams and rivers, increasing the frequency and severity of extreme flows (i.e. the upper and lower bounds of the flow duration curve) at both ends of the hydrological spectrum (i.e. floods and droughts). Historically, research effort has focused on the consequences of flooding and understanding of drought effects remains relatively poor (Lake, 2003; Lake, 2011).

Droughts are predicted to increase in prevalence globally (Handmer *et al*., 2012) and within the U.K. (Burke *et al*., 2010), where supra-seasonal droughts are expected to intensify across south-eastern England (Vidal & Wade, 2009) with potentially profound negative impacts upon aquatic biota (Lytle & Poff, 2004). The most noticeable response of rivers to hydrological drought is dewatering of the channel and associated effects on the availability and connectivity of aquatic habitat (Boulton, 1990). Dewatering can reduce habitat size, with implications for population survival during extreme conditions (White *et al*., 2016). Flow reduction during drought can also exacerbate the deposition of fine sediment in dewatering habitats (Wood & Petts, 1999). However, the prevalence of sedimentation depends on the extent of entrained sediment transportation in rivers, itself a reflection of catchment land use. Intensive arable farming is most likely to increase inputs into streams and rivers, although sediment can also be produced by industrial activities and bank re-profiling (Walling & Amos, 1999; Walling *et al*., 2003). The reduced thermal capacity

(Garner *et al*., 2014) and increased residence time (Mosley, 2015) of remnant water during drought may also lead to warming of stream habitats (Arismendi *et al*., 2013). The incidence and extent of warming depends largely on a suite of pressures such as atmospheric temperature, direct insolation and water volume (Webb *et al*., 2003; Webb & Zhang, 1999). Sedimentation may occur independently of drought (i.e. a temporary decline in flow velocity) whilst ecologically severe warming is unlikely to occur without prior dewatering. Thus dewatering may occur in combination with one or both of the above mentioned stressors to elicit a compound disturbance event. In future it is likely that extreme unprecedented hydrological droughts coupled with sedimentation (from increased land use intensity) and extreme water temperature fluctuations (from greater prevalence and severity of hot days) will occur more frequently (Arismendi *et al*., 2013), and thus it is imperative that we understand the importance of these cumulative stressors singly and in combination to inform mitigation priorities for water managers and conservationists.

To date most research has focused on structural responses to drought (due to a slow pull away from the Latin bionomial towards functioning responses) , and there has been a bias towards studies on macroinvertebrates as indicators of change owing to their ubiquity and sensitivity to change (e.g. Bogan *et al*., 2015; Boulton, 1990; Drummond *et al*., 2015; Ledger *et al*., 2012; Leigh *et al*., 2015; Lind *et al*., 2006 and Wright *et al*., 2002). There is evidence that drought can reduce both macroinvertebrate species richness (specifically shredder and predator groups) (Boulton, 2003; Dewson *et al*., 2007; Lake, 2003) and abundance (e.g. Wood & Petts, 1999) and further lead to marked turnover in the taxonomic composition of benthic assemblages, including the increase in abundance of small, multivoltine,

rapidly growing (*r*-selected) taxa (Ledger *et al*., 2012). In extreme cases drought has formed novel communities due to extirpation and replacement of larger bodied predators with smaller bodied taxa (Bogan & Lytle, 2011). Whilst knowledge of structural impacts is important, functional processes are also likely to be greatly impacted, yet have received less attention (Mustonen *et al*., 2016). Ecosystem processes yield a suite of services of societal value (Millenium Ecosystem Assessment, 2005; Palmer *et al*., 2009) such as public water supply, fisheries production (Heathwaite, 2010) and carbon sequestration (Palmer & Richardson, 2009) and may be threatened by climate change (Kundzewicz *et al*., 2008). A small number of studies have assessed drought impacts on key processes such as organic matter decomposition (Schlief & Mutz, 2009; Dewson *et al*., 2011; Schlief & Mutz, 2011) and both primary (Timoner *et al*., 2012; Magoulick, 2014; Stanley *et al*., 2003) and secondary (Chadwick & Huryn, 2007; Casanova *et al*., 2009) production, illustrating possible losses of functioning e.g. organic matter decomposition. Moreover, droughts could potentially modify whole-system processes such as stream metabolism (Carpenter *et al.*, 2011) and the associated production of greenhouse gases (Kosten *et al*., 2010), but research is scarce.

Organisms such as macroinvertebrates play crucial roles in the delivery of stream ecosystem functioning (Cummins & Klug, 1979; Graca, 2001; Petersen & Cummins, 1974) and drought effects on these biota may indirectly affect functional processes. Macroinvertebrates may be categorised into functional feeding groups (FFG) based upon their mode of feeding (Cummins, 1973). FFG biomass standing stock may be affected by both the total abundance of taxa but also the rate of secondary production – i.e. animal biomass accrual over time. Functional groups may be

disproportionately affected by stressors depending upon the relative sensitivity of constituent taxa (Ledger *et al*., 2011). For example, consumers such as shredders and predators with a large body size may be particularly vulnerable (Walters & Post, 2010), whilst shredders are known to also undertake compensatory respiration during drought in response to diminishing night time dissolved oxygen concentrations (Hughes, 1970), which may reduce oxygen demand further and increase mortality among sensitive taxa (Grant & Hawkes, 1982). Secondary production affects both the productivity of higher trophic levels and the removal rate of resources such as OM and living primary producers (Huryn & Wallace, 2000). Effects on these groups could have implications upon key functional processes within streams such as OM decomposition (Schlief & Mutz, 2009) and primary production (Ledger & Hildrew, 2000).

Primary production is a key process of benthic algae and macrophytes. Drought events have been shown to increase the rate of algal primary production (Suren *et al*., 2003), whilst others have reported strongly reduced algal abundance (Ledger *et al*., 2008; Timoner *et al*., 2012) which in turn is likely to reduce primary production. Macrophyte primary production can be affected by a range of abiotic variables (Lacoul & Freedman, 2006; Carr e*t al*., 1997) including temperature (Rasmussen *et al*., 2011) and water depth (Bucak *et al*., 2012). Macrophyte morphological adaptations during drought can alter leaf chlorophyll concentration and photosynthetic capacity (Nielsen & Sand-jensen, 1997). A long term data set study by Flynn *et al*. (2002) provides evidence that reduced flow can significantly reduce macrophyte biomass and percentage cover. Low flow reduced the cover of the dominant macrophyte *Ranunculus* spp. by 50% and smothered it in deposited

sediment (Wright, 1992). Drought can result in a loss of macrophytes and a succession of streambed flora towards a terrestrial community (Jones *et al*., 2012) which may have subsequent effects on streambed habitat and energy flow pathways (O'Callaghan *et al*., in prep).

Organic matter (OM) decomposition is a fundamental process in freshwater ecosystems (Petersen & Cummins, 1974). Disturbances such as drought reduce both microbial and macroinvertebrate mediated OM processing (Schlief & Mutz, 2009; Schlief & Mutz, 2011), as a result of a loss of shredders (Schlief & Mutz, 2009; Ledger *et al*., 2011) or modified animal behaviour in response to drought cues (Leberfinger *et al*., 2010) and / or alterations to the microbial community (Schlief & Mutz, 2009). In some circumstances, stressors typical of drought (e.g. reduced discharge, increased water temperature) can increase OM decomposition (Dossena *et al*., 2012; Ferreira & Canhoto, 2015; Friberg *et al*., 2013) or have little measurable effect (e.g. Death *et al.*, 2009).

Whilst the above patch scale descriptors are increasingly used to assess stressor impacts on ecosystem functioning in streams, studies conducted at the whole-system scale remain scarce. Stream metabolism, which describes the production and consumption of carbon within streams and rivers (Young, 2006), has been suggested for use as a proxy for overall stream health (Fellows *et al*., 2006; Mulholland *et al*., 2005; Young *et al*., 2008). For example, ecosystem respiration (ER) may correlate with other important functions such as gross primary productivity (GPP; Young *et al*., 2004) and decomposition (Rees *et al*., 2005; Perkins *et al*., 2010). Ecosystem respiration and gross primary productivity can be disproportionately affected by stress relative to one another (Logue *et al*., 2004;

O'Gorman *et al*., 2012; Yvon-Durocher *et al*., 2015) which may switch the system between a net carbon source and sink. Drought can affect benthic respiration specifically, through sedimentation (Shelly *et al*., 2015) and flow alteration (Rees *et al*., 2005). Jones (1995) found respiration was inversely related to sediment particle size in a desert stream, and Cardinale *et al.* (2002) found benthic respiration increased with habitat heterogeneity, indicating that substrate surface area and heterogeneity are important factors in regulating microbial colonisation. Organic matter may also significantly increase ER by increasing carbon availability (Hedin, 1990). Stream metabolism has been studied in the context of catchment disturbance (Mulholland *et al*., 2005) and physiochemistry and morphology (Hill *et al*., 1998), but most interest to date has focused upon warming (e.g. Yvon-Durocher & Allen, 2012; Williamson *et al*., 2015). Whole stream metabolism responses to hydrological extremes have typically only investigated flooding (e.g. Young & Huryn, 1996). However metabolism may also be affected by drought and has also been shown to react to river impoundments (Aristi *et al.,* 2014) and flow intermittency (Warfe *et al.*, 2014) in response to an increased accumulation of carbon (Acuña & Tockner, 2010). Flow restoration and reservoir releases have also shown to improve metabolic processes and increase production (Chester & Norris, 2006; Colangelo, 2007) further stressing the importance of flow in maintaining functional processes. Young & Collier (2009) reported stressed systems to become increasingly heterotrophic, suggesting that dried rivers may have reduced carbon sequestration capabilities. Metabolism (ER : GPP) describes the resource base of aquatic food webs (i.e. production (P) > respiration (R) = autotrophic; P<R = heterotrophic; e.g. Logue *et al*. 2004) reflecting the fate of carbon (i.e. net source / sink) of aquatic systems (Demars *et al*., 2011). To date, stream metabolism studies have focused

on single stressors (e.g. hydrological or morphological modification – usually warming) and the effect of compound disturbances are unknown. Clearly research investigating system metabolic responses to drought is in its infancy, but a thorough understanding is needed to predict changes in future ecosystem service delivery (Marcarelli *et al*., 2010).

Drought can be regarded as a compound event that exerts numerous physical stressors within streams and rivers (Rolls *et al*., 2012). Single stressors are likely to differ in their effect when combined with other stressors (ecological surprises; Christensen *et al*., 2006), and research is needed to identify potential interaction mechanisms in order to help predict and ameliorate future impacts (Ormerod *et al.*, 2010). The overall aims of this chapter were to: 1) determine how hydrologic drought affects key ecosystem processes at the patch (e.g. macroinvertebrate standing stock biomass, macrophyte primary production, leaf litter decomposition) and whole-system (e.g. metabolism) scale; and 2) determine the relative effect of individual constituent drought stressors (sedimentation, dewatering, warming) as well as their interactions on functional processes when applied singly and in-combination.

In this chapter, the effect of sedimentation, dewatering and warming on stream functioning were assessed using a series of outdoor mesocosms. Mesocosms have previously been used to study the effects of reduced flow (Schlief & Mutz, 2009), water loss (Ledger *et al*., 2012), sedimentation (Jones *et al*., 2015), warming (Dossena *et al*., 2012) and pollution (Grantham *et al*., 2012) on ecological processes and have been advocated for climate change research (Fordham, 2015) owing to their replicability, statistical power, and ease of abiotic variable

manipulability (Stewart *et al.*, 2013). The experiment reported here tested five key hypotheses: H_1 drought stressors will reduce macroinvertebrate FFG biomass, especially shredders (cf. Ledger *et al*., 2011). H_2 drought will reduce the growth of submerged macrophyte species, whilst emergent plant species will benefit increasing growth (Boulton, 2003). H_3 stressed treatments would be more heterotrophic than controls (cf. Young & Collier, 2009). H_4 drought stressors will increase the rate of benthic microbial respiration (Shelly *et al*., 2015). H_5 drought will have negative effects on macroinvertebrate organic matter processing (Schlief & Mutz, 2009) in part owing to a loss of shredder biomass.

3.3 METHODOLOGY

3.3.1 Study site

The research was conducted in a series of outdoor stream mesocosms located at Fobdown Farm (51°6'4"N, 1°11'13"W; Fig. 2.1 [section 2.21] for study site map), over a period of 42 days (April 2014 – May 2014) as part of the experiment described in Chapter Two. Forty channels were sited on a former watercress gravel bed adjacent to the Candover Brook. Abstracted groundwater (pH: 7.42; temperature: 10.25°C; conductivity: 963 µS) was supplied to a 220 L header tank which was then gravity fed to individual mesocosms through 12.5 mm inlet valves.

3.3.2 Experimental design

A 2 x 2 x 2 factorial experiment was conducted in the mesocosms to determine the ecological effects of three drought stressors – warming, dewatering, and sedimentation –applied singly and in combination (see Table 2.1, section 2.3.2).

Channels (horizontally-cut ribbed twin-walled sewage pipe [300 x 30 x 15 cm LWH] containing a 5 cm gravel depth at each 1 m end and a 1 cm depth in the central 1

m section) were seeded with macroinvertebrates, on day -1 (see Chapter 2). A fragment of *Ranunculus penicillatus* spp. *pseudofluitans* (Syme) S. D. Webster and *Berula erecta* (Huds.) Coville was planted in the top of each channel, including controls, to mimic habitat in local streams (see Fig. 3.1a). Plant fragment fresh weight (*fW* g) was recorded prior to planting in order to calculate relative growth rate for the duration of the experiment.

One leaf pack with coarse (5 mm) mesh, and one leaf pack with fine (500 µm) mesh (see Fig. 3.1b) were implanted in each channel on day 0 (total 40 fine mesh bags, 40 coarse mesh bags). Leaf packs contained freshly abscised alder (*Alnus glutinosa*) leaves that were collected during November 2013 and air dried after Ferreira & Canhoto (2015). Three grams of air-dried leaves were added to mesh bags (i.e. leaf pack). Fine mesh bags excluded most macroinvertebrates (see Section 3.5) and thus provided a better representation of microbial decomposition.

3.3.3 Sample processing

Diel oxygen (DO; mg L^{-1}) data for each channel were recorded over days 34 - 38 using miniDOT dissolved oxygen loggers (Precision Measurement Engineering (PME) Inc., California, USA). Loggers were incubated in each channel for 24 hours. Photosynthetic active radiation (PAR; µmol m^{-2} s^{-1}) was also recorded during the DO logging period using a LI-193 Spherical Quantum Sensor and LI-1400 datalogger (LI-COR, Lincoln, Nebrasca, U.S.A.).

At the end of the experiment (day 42), macroinvertebrates were collected (1 sample per channel) using a small Surber sampler (0.08 m^2) and preserved in 70% industrial methylated spirit (IMS). Macrophytes were harvested (whole plant including roots) sorted from debris, and weighed (fresh weight; *fW* g). A subsample

of representative leaves (n = 5) from each specimen of *R. pseudofluitans* and *B. erecta* was also collected for chlorophyll analysis (see below). Plant and leaf samples were stored in the dark at -18 °C. Leaf packs were removed from channels and frozen. One representative sample of the benthic substratum was collected from the centre of each channel using equal effort (encompassing any gravel and finer material from the top two centimetre depth of the substratum) and stored in a refrigerator in the dark for a benthic respiration experiment (outlined below).

One water sample was collected from the central pool (approximately 180 cm from channel header) of each channel, and the header tank, on day 42 for phosphate (PO_4^{2-}) and nitrate (NO_3^-) analysis. Stream water was filtered through a 0.22 μm pore size filter (PES, ANR2522C) using a 50 ml syringe and subsequently frozen. Concentrations of PO_4^{2-} and NO_3^- were measured using a segmented flow auto analyser (Skalar, type 5000, Skalar Analytical B.V, The Netherlands) and standard colorimetric techniques following Acuña *et al*. (2005). The limit of detection (LOD) and precision for PO_4^{2-} and NO_3^- was 0.2 μmol L^{-1} ± 1%. A suite of standards were made (0.25 μm, 0.5 μm, 2 μm, 10 μm and 20 μm) for PO_4^{2-} and NO_3^- to produce a calibration curve. The 2 μm standard was used to assess drift of the instrument. A 5 μm multi standard solution was created to compare against a spiked ground water sample to ensure drift correction was adequate during the day long analysis. Double deionised water was used as a blank.

Macroinvertebrate functional feeding groups and biomass estimation

In the laboratory, macroinvertebrates were sorted and identified to the lowest practicable taxonomic unit (usually species). Individual body length and head width of a representative number of individuals (≥10 taxa channel^{-1}) were measured to the nearest 0.1 mm using a Carl Zeiss W-Pl 10x / 23 graticule eyepiece for each taxon. Lengths were converted to individual biomass (mg) using published length-mass equations (Table B1, Appendix B). Taxa were assigned to one of five FFG's (collector, filterer, grazer, predator or shredder) and biomass estimates were subsequently calculated. Sampled individual numbers were up-scaled to m^2 to calculate FFG biomass and total macroinvertebrate biomass (mg m^{-2}) per channel.

Macrophyte primary production

The total biomass (g *fW*) of macrophytes at the start and end of the experiment was used to calculate their relative growth rate (Equation 1) in each replicate mesocosm over the experimental period (42 d).

$$\text{RGR } (\hat{r}_2) = \frac{\ln(W2) - \ln(W1)}{t2 - t1} \qquad \text{(Eq. 1)}$$

where relative growth rate (RGR) represents *fW* g day^{-1} of macrophyte growth, and ln(W) represents log transformed fresh weight at the start (W1) and end (W2) of the experiment. *t* represents time.

The photosynthetic capacity of macrophytes was determined by chlorophyll extraction of subsampled macrophyte leaves using the methods by Lichtenthaler &

Buschman (2005) in order to determine stress effects upon plant condition and primary production potential. $MgCO_3$ was added to 50 mg of bored, dried leaves and 100% acetone was then used to extract chlorophyll. The mixture was ground, placed into a graduated centrifuge tube, and centrifuged for five minutes in a cooling table top centrifuge. A 1 ml aliquot was extracted from each centrifuge tube and placed within a 1 cm path-length cuvette for wavelength absorption analysis within a Jenway 6305 UV-VIS spectrophotometer (Bibby Scientific, Stone, UK). 662 nm was used for chlorophyll a, and 750 nm was used to account for turbidity. Absorption readings from the UV-VIS spectrophotometry were used to calculate chlorophyll a (µg mL^{-1}; Equation 2).

$$Chl\ a = 11.24\ x\ A_{662} - 2.04\ x\ A_{645} \quad \text{(Eq. 2)}$$

where A662 was light absorbance readings at 662nm. These values were then converted to mg g^{-1} of leaf.

Organic matter decomposition

Leaf packs were defrosted, sorted from other non-leaf litter, oven dried, weighed, placed into a muffle furnace at 450 °C and reweighed to establish AFDM, following Tolkkinen *et al*. (2015). In addition to AFDM correction, transportation (leaf mass loss following transportation to the field), leaching (leaf mass loss following 24 hours in deionised water) and moisture (difference between oven dried and air dried leaves) corrections were made following Bruder *et al*. (2011). The decay coefficient

'*k*' was calculated (Equation 3) to represent leaf mass loss, following Petersen & Cummins (1974).

$$-k = \frac{-\ln((dm_r \times a) / dm_i \times mlat}{d} \quad \text{(Eq. 3)}$$

where dm_i is the initial mass of leaves (~3 g) and dm_r is the mass remaining following the experiment. The correction '*m*' is moisture, '*l*' is leaching, '*a*' is AFDM and 't' is transportation loss. '*d*' represents the number of days of the experiment.

The two leaf pack types enabled three rates of leaf litter decomposition to be calculated: Ktotal (leaf mass loss from coarse packs), Kmicrobe (leaf mass loss from fine packs) and Kinvert (Ktotal - Kmicrobe).

Ecosystem metabolism

Gross primary productivity (GPP) and ecosystem respiration (ER) were calculated using the BAyesian Single-station Estimation (BASE) method (Grace *et al*., 2015), which uses the statistical software R (R Core Development Team) which in turn calls the model and invokes OpenBUGS version 3.2.3 (Lunn *et al*., 2009) to run. BASE uses Bayesian estimation to solve Equation 4 over a 24 hour period.

$$\Delta[O_2]_i / \Delta t = A I_i^p - R \left(\theta^{(T_i - T)}\right) + k_{o2} \left(1.0241^{(T_i - T)}\right) D_i \quad \text{(Eq. 4)}$$

where AI_i^p represents primary production, hereafter *P*. (*A* = constant; I = incident light intensity; *p* = exponent representing a producers ability to utilise incident light; *i* = diel profile time increments. *R* = rate of ecosystem respiration; T_i = water temperature; T = mean 24 hour temperature; *D* = oxygen saturation surplus and k_{O2} = reaeration coefficient.

Thus BASE provides an indirect modelling approach that incorporates k_{O2} as a parameter with *P* and *R* to fit the raw diel DO curve (Grace et al., 2015).

Net production (NEP), which represents total carbon available (Lovett *et al.*, 2006) was additionally calculated by deducting ER from GPP.

Benthic respiration

A subsample (mean dry weight = 3.35 ± 0.07 g) of the refrigerated benthic substrate collected from each replicate mesocosm was added to dry pre-weighed gas tight vials, along with 6 ml of groundwater used to supply the mesocosm channels, in order to mimic the physicochemistry of the channels during sediment collection. Sediment within the vials were incubated within a 15 °C constant temperature room on a reciprocating shaker table at 85 RPM. An additional six vials were added to the analysis: three contained groundwater only and three contained gas only. Of the latter three, two contained air which were used to ensure that peaks were being detected, and the remaining vial contained a CO_2 / CH_3 / N_2O certified standard (3699 / 100 / 100 ppm respectively, BOC, special gas mix), used as the calibration standard. Gas chromatography was conducted using a gas chromatograph (Agilent

6890N, Agilent Technologies, Berkshire UK) using a flame ionisation detector (FID). ‘GC Chemstation’ (revision A.10.02) software (Agilent Technologies, U.S.A.) was used for peak analysis. CO_2 was identified based upon retention time (approx. 2.5 minutes) of the standard gas mix. The FID process was repeated an additional three times until CO_2 production had plateaued. The slope of the CO_2 production curve was subsequently calculated, and corrected for time to determine CO_2 production, measured as CO_2 g h^{-1}.

Figure 3.1. **Photographs of the mesocosm channels**. Illustrating (a) newly planted macrophytes (day -27) and (b) freshly transplanted leaf packs (day 0).

a)

b)

3.3.4 Data analysis

For all measured responses, variable distributions were analysed using QQ plots, and outliers were examined using box plots. Normal distribution was statistically tested using Shapiro-Wilk tests and homogeneity of variance was tested using the Bartlett test. Biological data were log-transformed, if necessary, to improve normality and homoscedasticity, following methods by Townsend *et al*. (2008) and recommendations by Ives (2015).

A three-way analysis of variance (ANOVA) was conducted to test for main effects and their interactions of stressors on macroinvertebrate biomass, primary production, OM decomposition, ecosystem metabolism (ER, GPP, GPP : ER) and benthic respiration. The ANOVA model tested for significance of individual stressors (i.e. main effects), and for the significance of interaction effects of stressors in combination ($P < 0.05$).

Significant interactions detected by the three way ANOVA were subsequently followed up using Tukey HSD *post-hoc* tests, as well as means testing of control vs. treatments, and interaction plot visualisation, to detect significant differences between treatment means. Where stressors had significant main effects *and* interactions, interpretation of main effects should be carefully considered (Piggott *et al*., 2015). Where this occurred, main effects were only considered when their effect size was greater than that of the interaction, following methods by Quinn & Keough (2002). Bonferroni was not used owing to the smaller number of tests for each hypothesis, relative to Chapter 2.

All data exploration, visualisation and analyses were conducted using R, version 3.2.0 (R Core Development Team, 2015).

3.4 RESULTS

In total 9610 individual macroinvertebrates spanning 44 taxa were collected from the channels at the end of the experiment (Table A1, Appendix A). Of these, biomass estimates were derived for 2357 individuals. Mean total biomass of macroinvertebrates (per channel) was 2889 mg m^{-2}, with grazers, shredders, collectors, predators and filterers contributing 71.12, 16.07, 7.45, 5.31 and 0.04% of this total biomass, respectively (Fig. 3.3).

There were no statistically significant main effects (i.e. stressors acting individually) of the stressors on total macroinvertebrate biomass ($P > 0.05$; Figs. 3.3-3.4; Table 3.1), but an interaction between temperature and sediment was significant (three way ANOVA, $P < 0.019$; Fig. 3.4a; Table 3.1): Total biomass was significantly greater when warming and sediment application were combined (WS) when compared with warming (W) alone (Tukey, $P = 0.031$). A significant interaction between temperature and dewatering (three way ANOVA, $P < 0.001$; Fig. 3.4b; Table 3.1) revealed collector biomass was significantly greater in warmed (W) channels and dewatered (D) channels than controls (Tukey, $P = 0.005$ and 0.006, respectively) whereas the two stressors combined (warmed and sediment; WS) were not statistically different to the control ($P > 0.05$). There was a main effect of warming with grazer biomass greater in warmed channels than non-warmed channels (three way ANOVA, $P = 0.021$; Fig. 3.4c; Table 3.1). Effects of stressors on predator biomass were not statistically significant ($P > 0.05$; Fig. 3.4d). A main effect of dewatering revealed shredder biomass was suppressed in dewatered channels (three way ANOVA, $P = 0.036$; Fig. 3.4e; Table 3.1). Filterer biomass was

excluded from the global test due to a high proportion of missing values (absence of individuals) across the dataset.

Drought treatments had contrasting effects on the relative growth rate and photosynthetic capacity of the two macrophyte species (Table 3.2; Fig. 3.5). A main effect of temperature and dewatering revealed that *R. pseudofluitans* RGR was suppressed in warmed channels (three way ANOVA; $P = 0.032$; Fig. 3.6a; Table 3.2) and dewatered channels (three way ANOVA; $P < 0.001$; Fig. 3.6a; Table 3.2). Sediment and dewatering interacted ($P = 0.035$; Table 3.2; Fig. 3.6a) to reduce *R. pseudofluitans* RGR when sediment application and dewatering were both applied (SD; Tukey HSD, $P < 0.001$) when compared to sediment (S) alone. A main effect of temperature on *B. erecta* RGR was dropped (see methods: data analysis) because of an interaction between temperature x sedimentation x dewatering (three way ANOVA, $P = 0.009$; Fig. 3.6b; Table 3.2) which had a greater effect size in comparison to the main effect. Subsequent means testing revealed a significant difference between control (C) and warmed (W) treatments (Welche's t test; $P = 0.014$), C and sediment applied (S) treatments (Welche's t test; $P = 0.021$) and C and all-three stressor (WSD) treatments (Welche's t test; $P = 0.007$). The resultant three way interaction was a two way interaction between warming and sediment that was in turn determined by the level of a third stressor, dewatering (Figure B1, Appendix B). A main effect of dewatering on *R. pseudofluitans* leaf chlorophyll a concentration revealed chlorophyll concentrations were significantly greater in dewatered channels (three way ANOVA; $P = 0.009$; Fig. 3.7; Table 3.2) whilst a main effect of both temperature (three way ANOVA; $P = 0.027$; Fig. 3.7; Table 3.2) and sediment (three way ANOVA, $P = 0.047$; Fig. 3.7; Table 3.2) increased *B. erecta*

leaf chlorophyll a concentration in all warmed (W) and sediment applied (S) treatments, respectively.

Diel oxygen curves varied through time in all treatments reflecting diel trends in PAR (Figs. 3.8-3.11). Dewatered treatments typically showed greater day time and lower night time DO concentrations relative to non-dewatered channels, suggesting greater rates of ER and GPP. ER and GPP typically responded similarly to treatments (Figs. 3.12a; 3.12b). No statistically significant effects of stressors on ER were detected (P >0.05), whereas a main effect of dewatering on GPP (three way ANOVA; P = 0.048; Fig. 3.12b; Table 3.3) revealed elevated GPP in dewatered channels. Stressors had no statistically significant effects on ER:GPP (P >0.05), which were >1 (Fig. 3.13a) in all treatments (except in the warmed and sediment applied and dewatered [WSD] treatment) indicating overall heterotrophy in those channels. Greater negative NEP values were found in warmed treatments (i.e. W, WS, WD; Fig. 3.13b), indicating heterotrophy was greater in warmed than ambient treatments. In the benthic substrate respiration experiment, a main effect of sediment was detected on rate of respiration (three way ANOVA, P <0.001; Fig. 3.14; Table 3.3) which demonstrated respiration rates were greater in sediment applied (S) treatments.

A main effect of sediment on Ktotal (three way ANOVA; P =0.001; Fig. 3.15; Table 3.4) revealed Ktotal was reduced in sediment applied (S) treatments. A main effect of sediment on Kmicrobe (three way ANOVA, P <0.001; Fig. 3.15; Table 3.4) also revealed that Kmicrobe was reduced in sediment applied (S) channels, although positive decay coefficient values were detected in sediment applied treatments. No

statistically significant ($P > 0.05$) effects of stressors were detected on Kinvert decay.

The concentrations of both nitrate and phosphate recorded in each channel at the end of the experiment are presented in Table 3.5. Concentrations of phosphate were below the limit of detection (LOD) in all non-dewatered channels (i.e. C, S, W and WS) and in the header tank (i.e. freshly abscised groundwater). However, P accumulated in dewatered channels. P concentration was greatest in the WSD treatment (3.98 µM). In contrast, nitrate was greater in groundwater (294.24 µM measured in the header tank) and in all non-dewatered treatments, whereas in all dewatered treatments (i.e. D, SD, WD, WSD), nitrate was much lower than the groundwater source, and lowest in the WSD treatment (120.59 µM; 59% lower relative to control), suggesting increased denitrification in the dewatered channels.

Figure 3.2. **Photographs taken of two contrasting mesocosm treatments**. (a) WSD (all three stressor) treatment showing abundant filamentous algae and (b) untreated control treatment, at the end of the experiment.

a)

b)

Figure 3.3. **Macroinvertebrate standing stock.** Treatment responses grouped by functional guilds (arranged in ascending order of proportional biomass from filterers to grazers). Treatments defined by codes, where C = control, W = warmed, S = sediment and D = dewatered. Bars represent mean biomass ± 1SE. Note filterers not visible on this scale, but very small filterer biomass was detected.

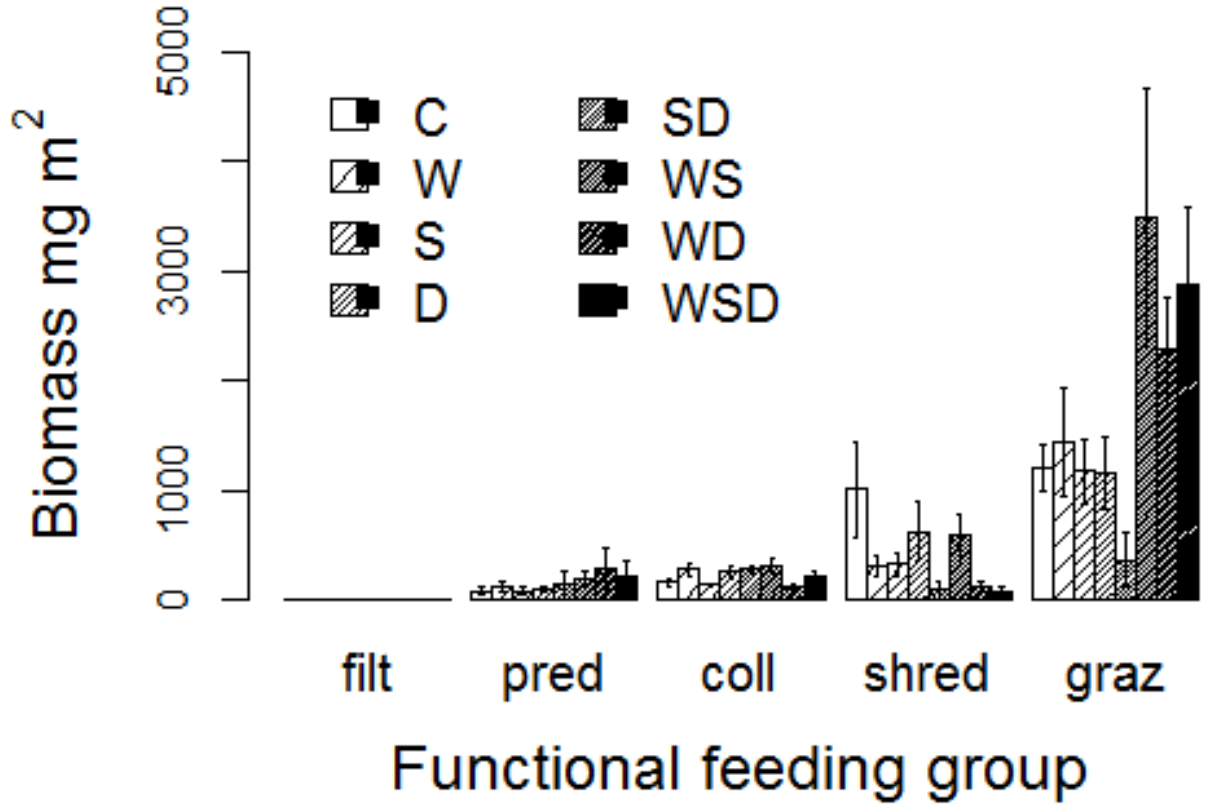


Figure 3.4. **Mean (± 1SE) functional feeding group biomass responses to treatments**. Graphs show a) total biomass of macroinvertebrates among treatments and b-e) biomass of different functional guilds. Treatments defined by treatment codes, where C = control, W = warmed, S = sediment and D = dewatered. Bar tone denotes number of stressors applied (white = 0; light grey = 1; dark grey = 2; black = 3). N.B different y axis limits.

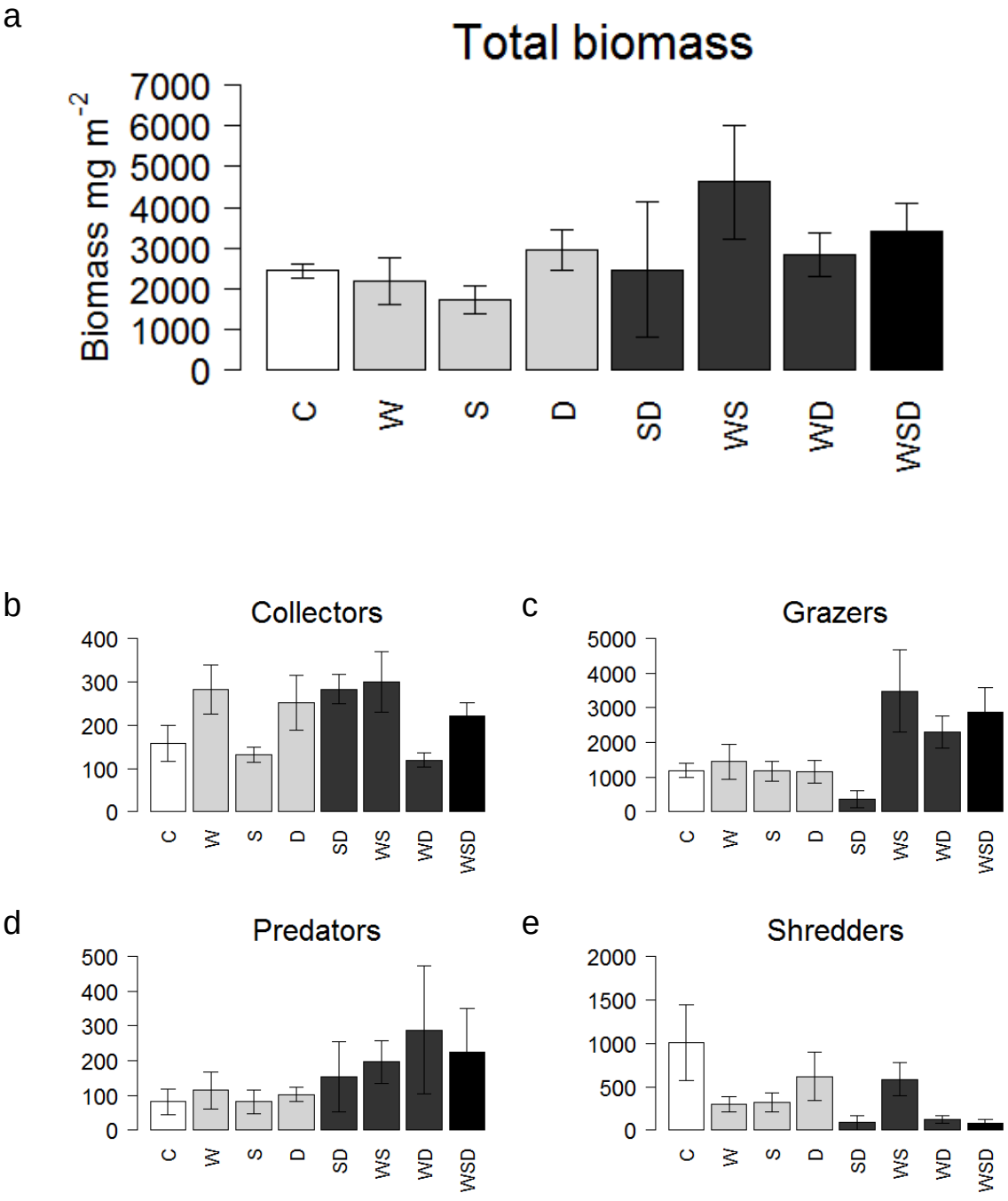


Table 3.1. **Three way ANOVA summary results illustrating stressor effects on functional feeding group biomass***. Values show *P* value responses. Significant values (*P* <0.05) shown in bold. 'Temperature', 'sediment' and 'dewatering' show main effects. Other columns indicate interaction effects.

Dependent variable	Temperature	Sediment	Dewatering	Temperature x sediment	Temperature x dewatering	Sediment x dewatering	Warming x sediment x dewatering
Collector	0.467	0.306	0.582	0.118	**<0.001**	0.397	0.215
Grazer	**0.021**	0.539	0.983	0.223	0.246	0.690	0.296
Predator	0.103	0.594	0.245	0.287	0.252	0.760	0.621
Shredder	0.512	0.058	**0.036**	0.126	0.189	0.671	0.314
Total biomass	0.100	0.872	0.364	**0.019**	0.450	0.615	0.361

*See Table B2, Appendix B for three way ANOVA model outputs

Figure 3.5. **Photographs of harvested macrophytes at the end of the experiment**. Images show a) *Berula erecta* from a warmed channel, with turgid, green foliage, b) *Berula erecta* from a control untreated channel with yellowed foliage, c) *Ranunculus pseudofluitans* from a control channel (large individuals, long leaves) and d) *Ranunculus pseudofluitans* from a dewatered treatment (small individuals, short leaves, rigid).

a)

b)

c)

d)

Figure 3.6. **Mean (± 1SE) Relative Growth Rate (RGR) of two contrasting macrophyte taxa**. *Ranunculus pseudofluitans* a) and *Berula erecta* b). Treatments defined by codes, where C = control, W = warmed, S = sediment and D = dewatered. Bar tone denotes number of stressors applied (white = 0; light grey = 1; dark grey = 2; black = 3)

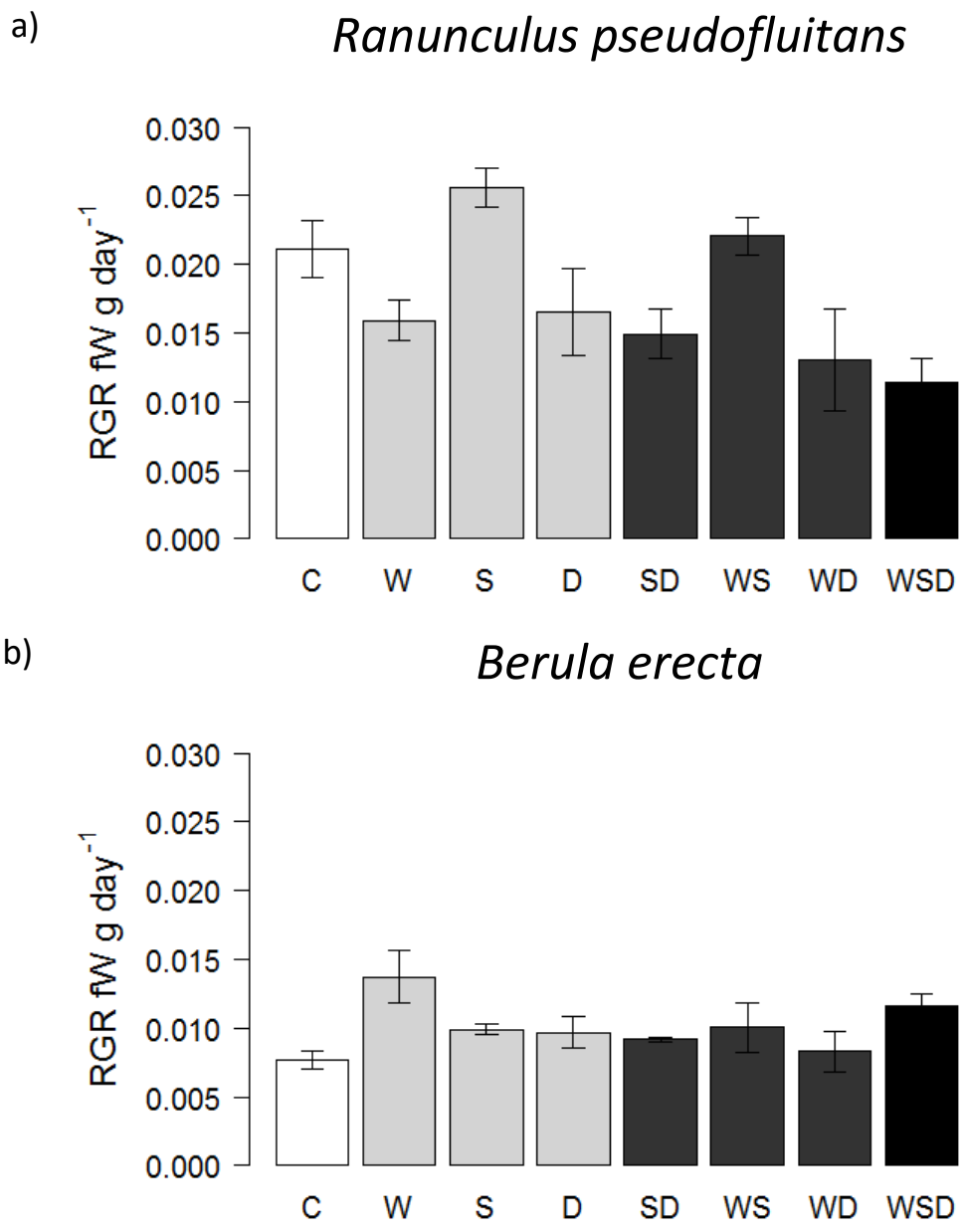


Table 3.2. **Three way ANOVA summary results illustrating stressor effects on macrophyte growth and health parameters***. Significant values (*P* <0.05) shown in bold. 'Temperature', 'sediment' and 'dewatering' show main effects. Other columns indicate interaction effects.

Dependent variable	Temperature	Sediment	Dewatering	Temperature x sediment	Temperature x dewatering	Sediment x dewatering	Warming x sediment x dewatering
Ranunculus RGR	**0.018**	0.252	**<0.001**	0.808	0.792	**0.035**	0.797
Berula RGR	**0.032**	0.799	0.555	0.458	0.175	0.239	**0.009**
Ranun. chl a	0.938	0.957	**0.009**	0.981	0.679	0.250	0.624
Berula chl a	**0.027**	**0.047**	0.956	0.835	0.701	0.223	0.078

*See Table B3, Appendix B for three way ANOVA model outputs

Figure 3.7. **Mean macrophyte leaf chlorophyll concentration (mg g $^{-1}$; ± 1SE) across treatments**. Values to the right and left of vertical dashed line indicate treatments that are dewatered and not dewatered, respectively. Treatments defined by codes, where C = control, W = warmed, S = sediment and D = dewatered.

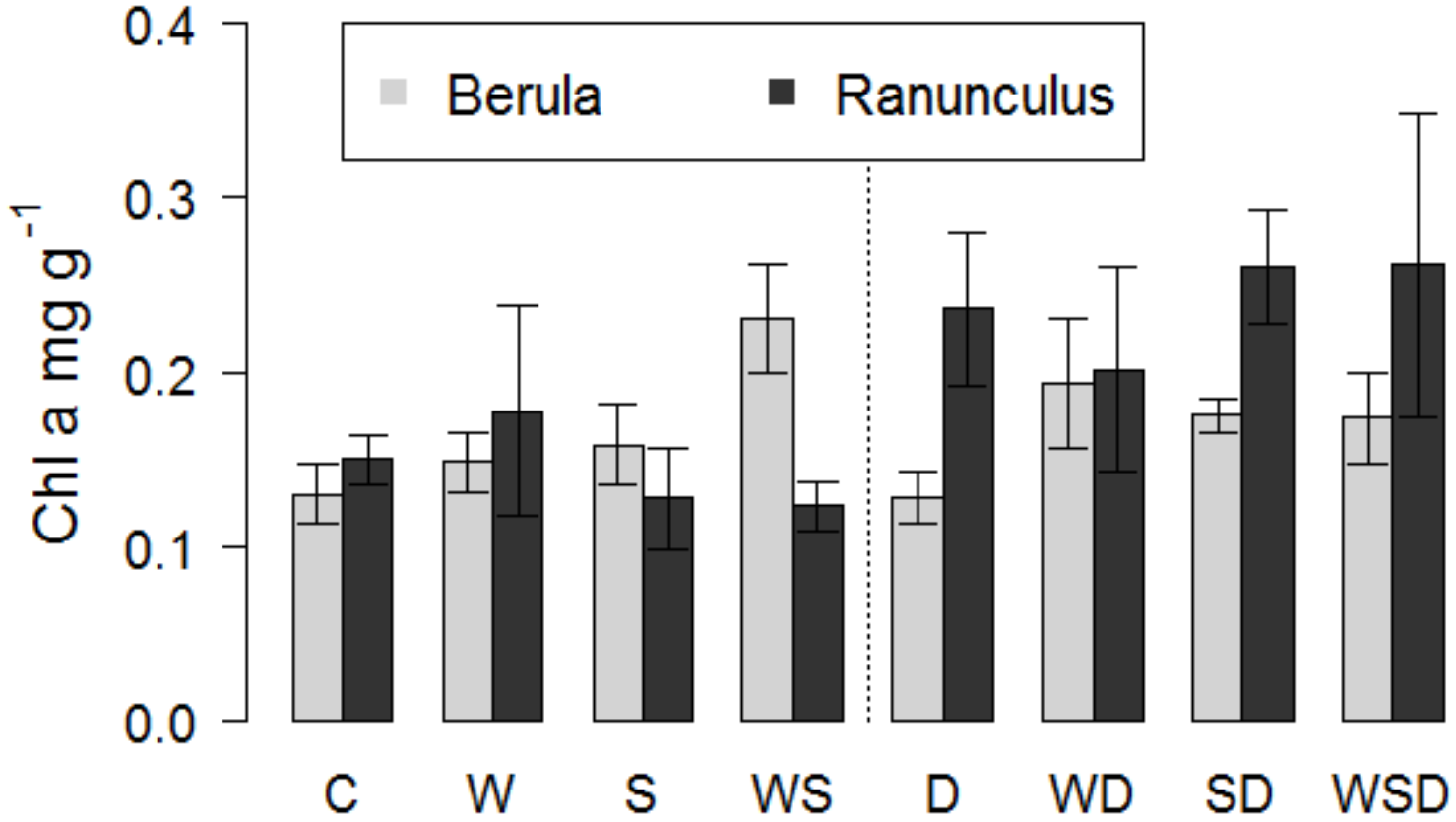
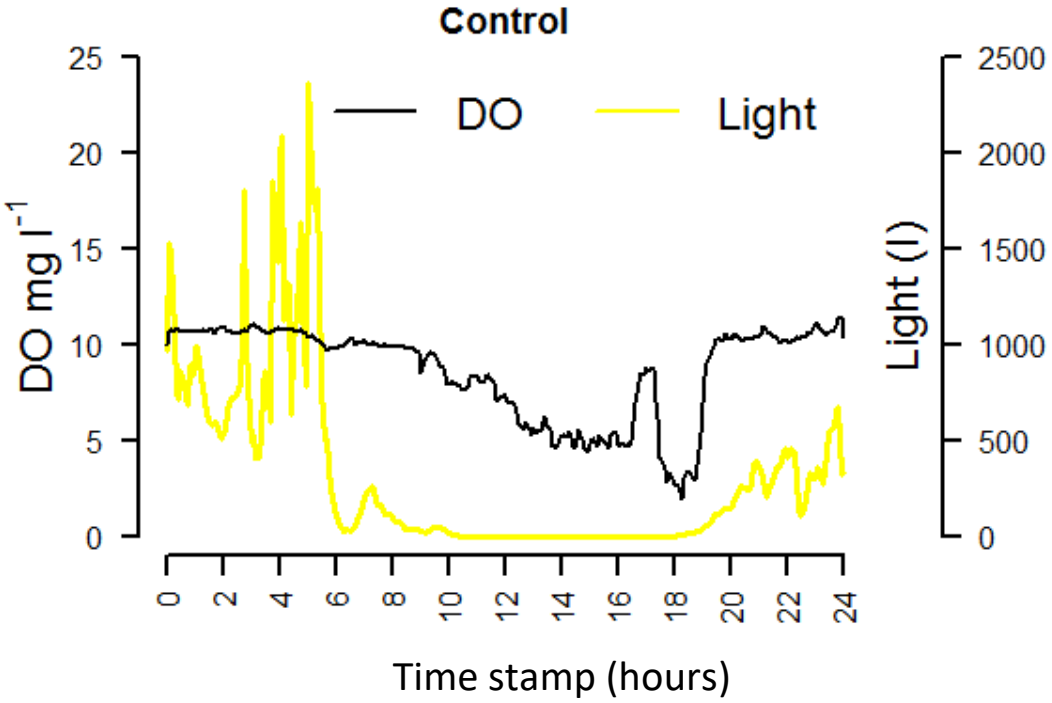


Figure 3.8. **Dissolved oxygen and light (PAR) diel curves**. DO (mg l^{-1}) time series for 3rd June 2016 for one single replicate from each treatment. Showing a) control (C) and b) dewatered (D) treatments.

a)



b)

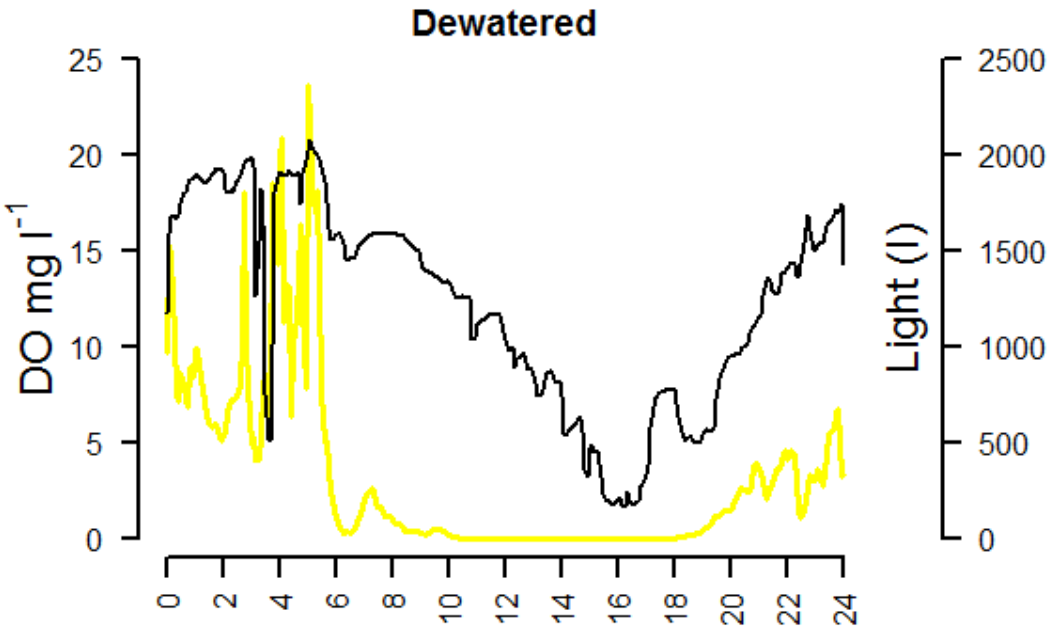
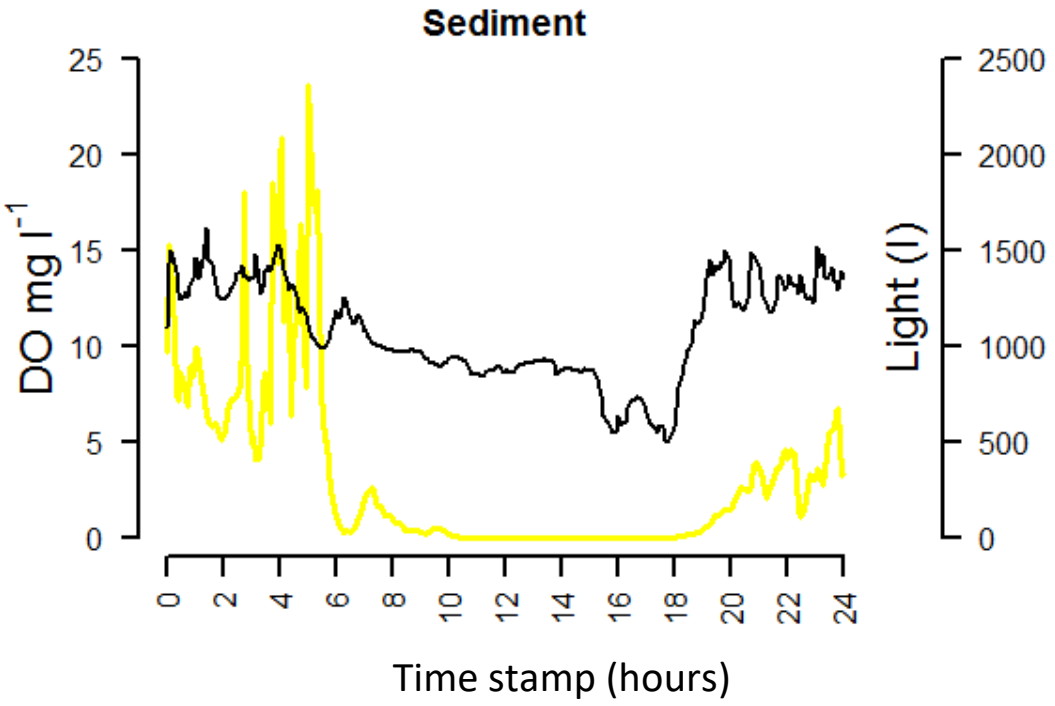


Figure 3.9. **Dissolved oxygen and light (PAR) diel curves**. DO (mg l^{-1}) time series for 3rd June 2016 for one single replicate from each treatment. Showing a) sedimented (S) and b) warmed (W) treatments.

(a)



(b)

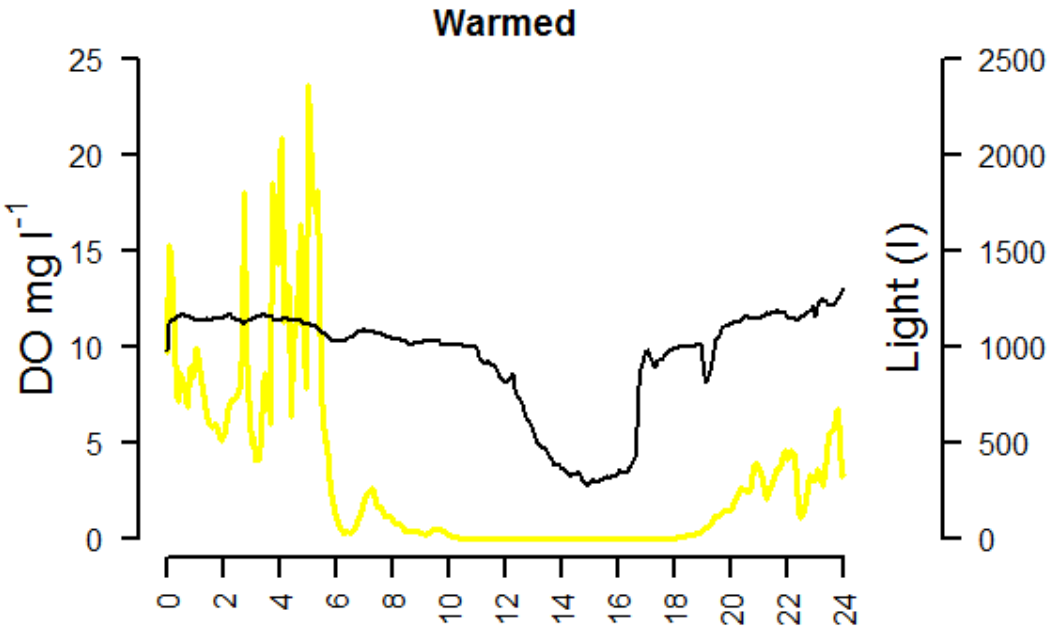
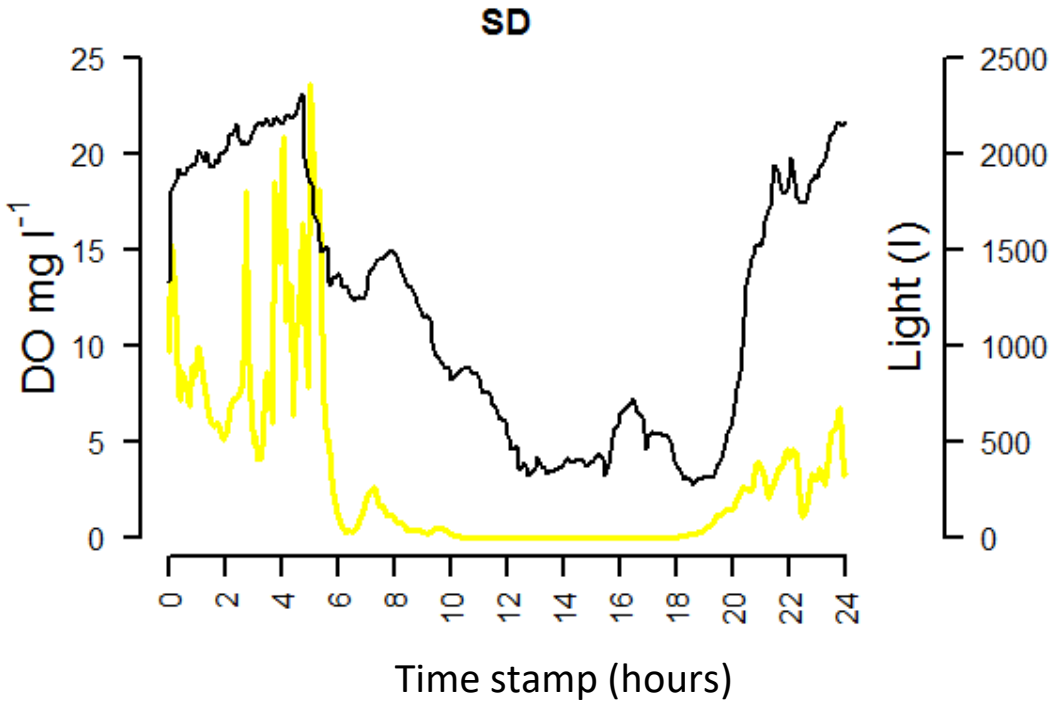


Figure 3.10. **Dissolved oxygen and light (PAR) diel curves**. DO (mg l^{-1}) time series for 3rd June 2016 for one single replicate from each treatment. Showing a) sedimented and dewatered (SD) and b) warmed and dewatered (WD) treatments.

(a)



(b)

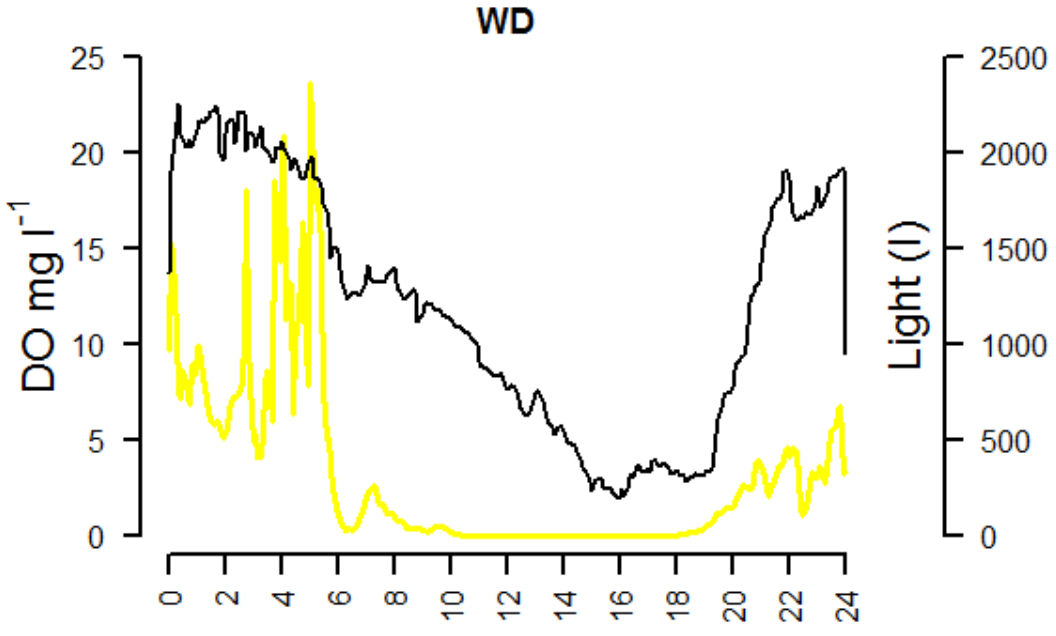
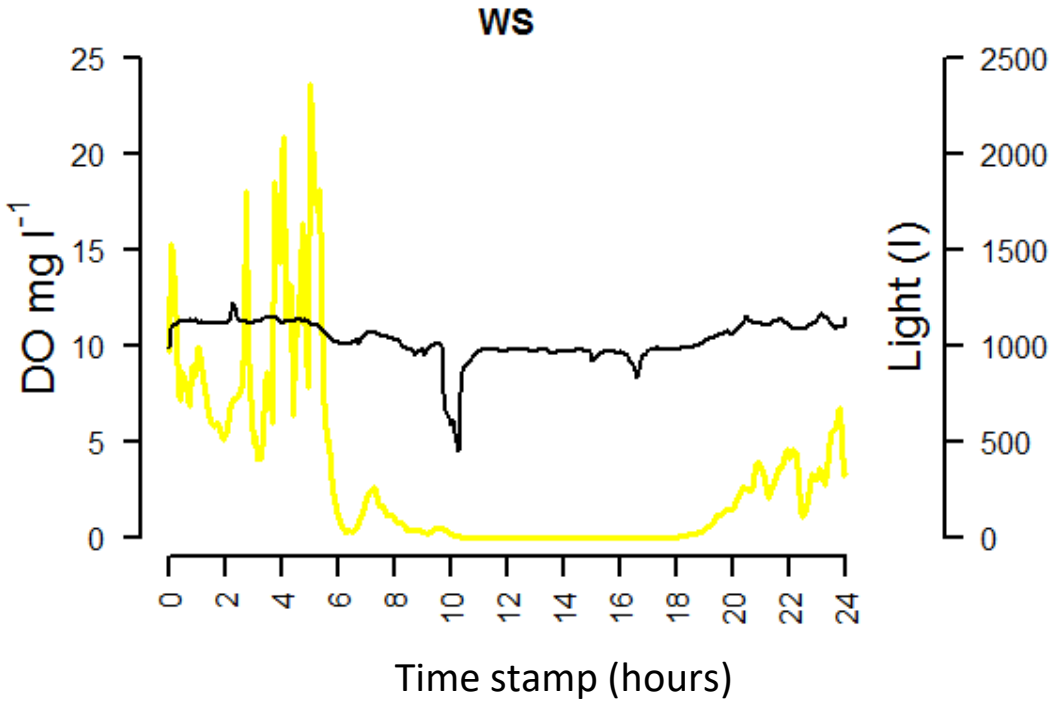


Figure 3.11. **Dissolved oxygen and light (PAR) diel curves**. DO (mg l^{-1}) time series for 3rd June 2016 for one single replicate from each treatment. Showing a) warmed and sedimented (WS) and b) warmed and sedimented and dewatered (WSD) treatments.

(a)



(b)

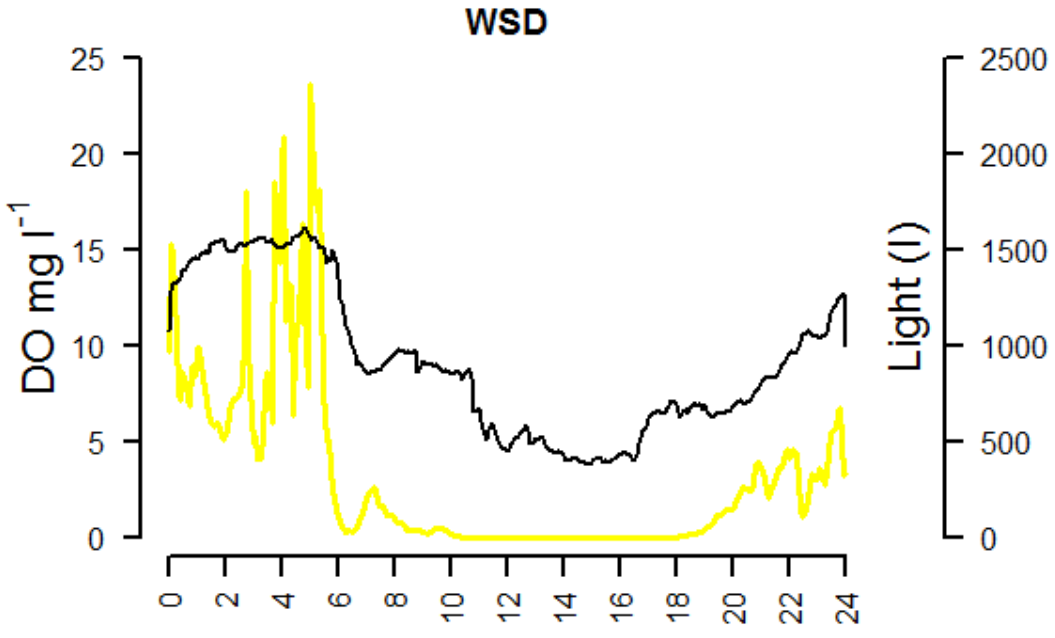
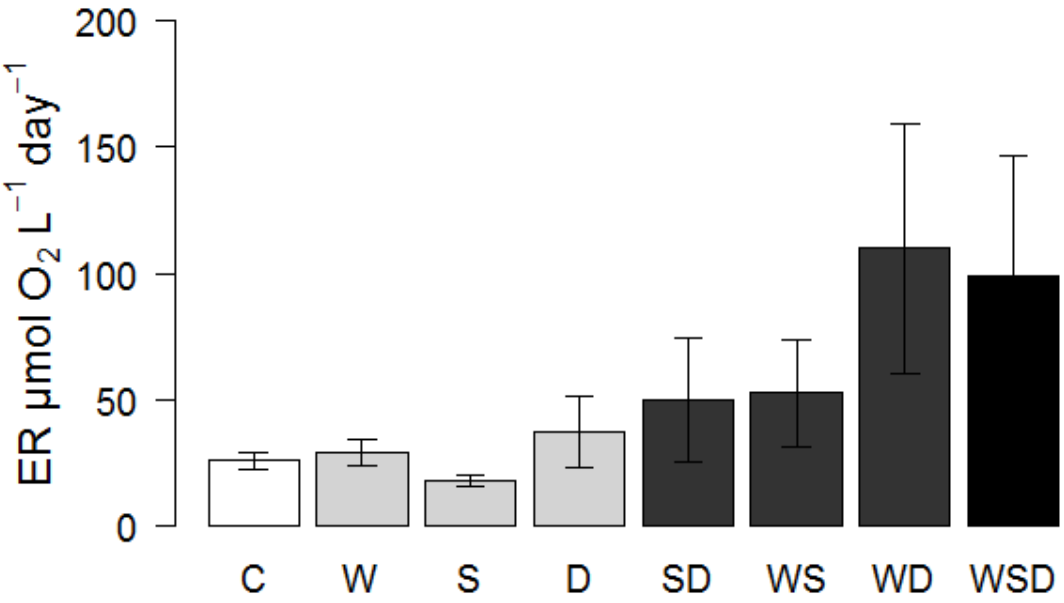


Figure 3.12. **Ecosystem metabolism responses among treatments**. Showing a) Ecosystem Respiration and b) Gross Primary Productivity. Bar tone denotes number of stressors applied (white = 0; light grey = 1; dark grey = 2; black = 3). Data are mean values ± 1SE. Treatments defined by codes, where C = control, W = warmed, S = sediment and D = dewatered.

a)



b)

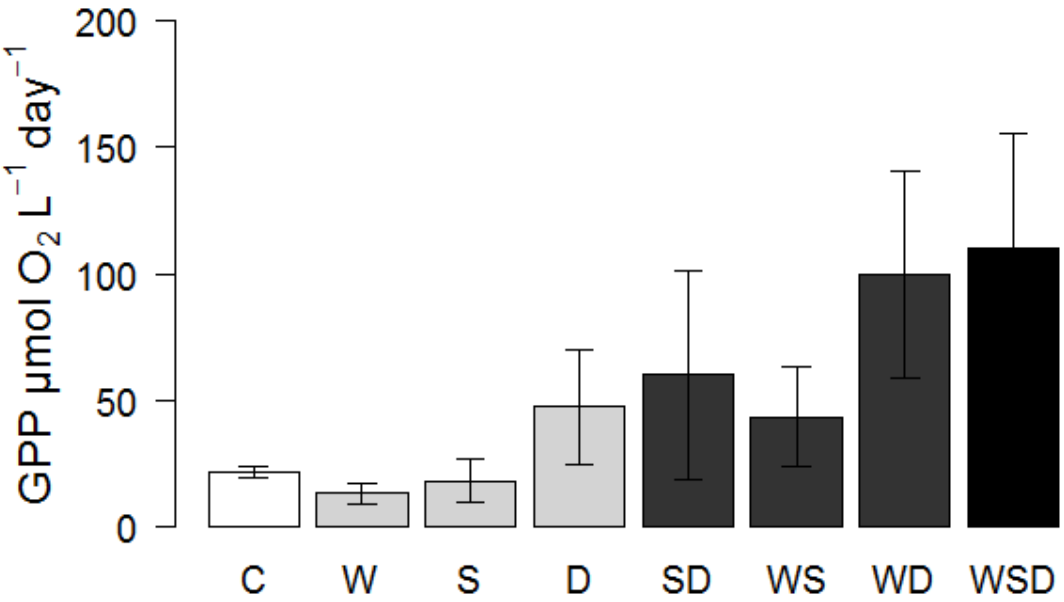
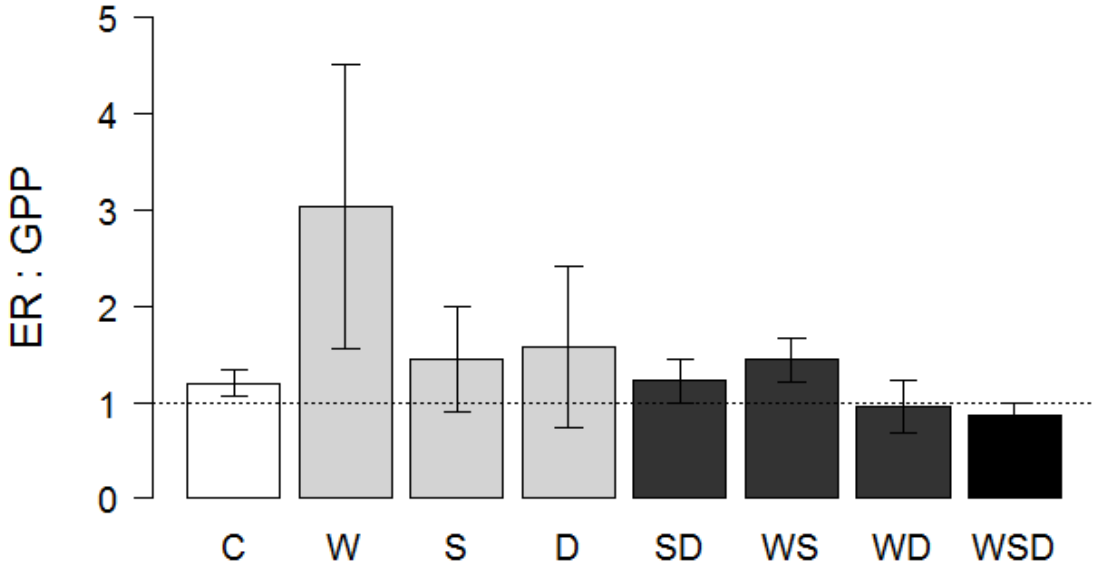


Figure 3.13. **Ecosystem metabolism responses among treatments**. Plots showing a) ER:GPP ratio and b) Net Ecosystem Production. Bar tone represents number of stressors applied (white = 0; light grey = 1; dark grey = 2; black = 3), except in (b) where bars are grouped by warming (separated by vertical dashed line). Data are mean values ± 1SE. Treatments defined by codes, where C = control, W = warmed, S = sediment and D = dewatered.

a)



b)

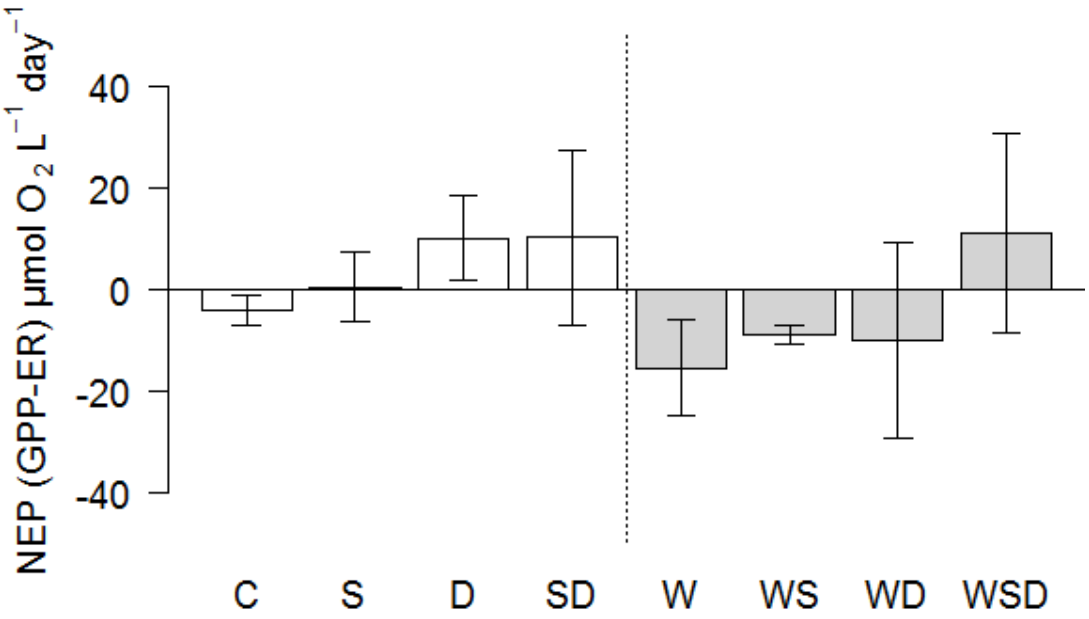


Figure 3.14. **Ecosystem metabolism responses among treatments**. Showing benthic respiration (laboratory CO_2 production). Bar colour represents number of stressors applied (white = 0; light grey = 1; dark grey = 2; black = 3). Data are mean values ± 1SE. Treatments defined by codes, where C = control, W = warmed, S = sediment and D = dewatered.

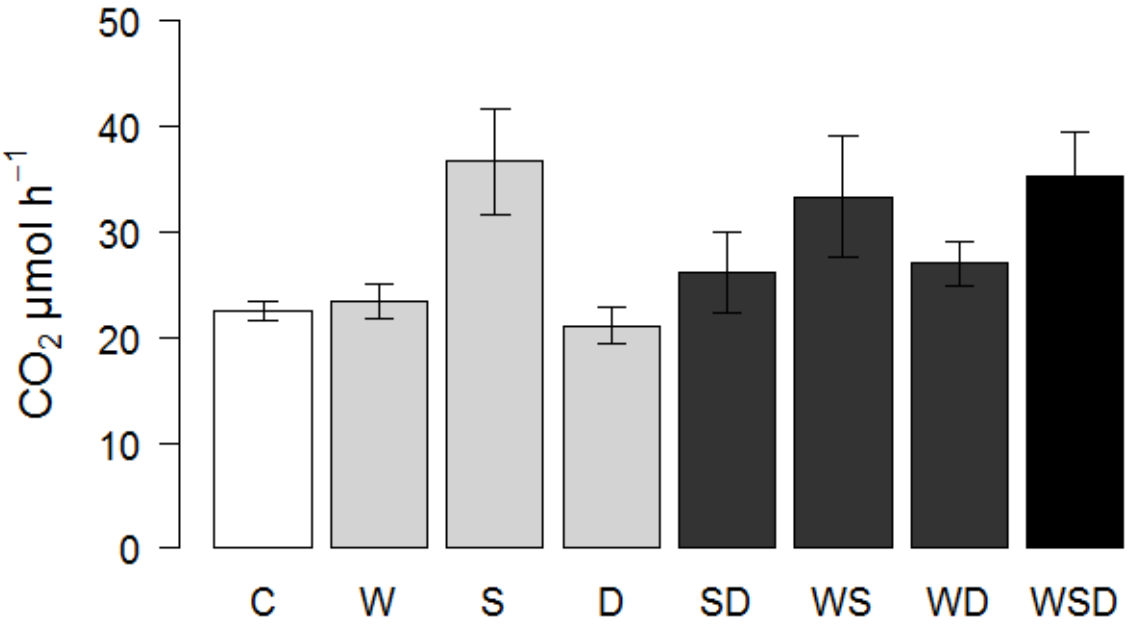


Table 3.3. **Three way ANOVA summary results illustrating stressor effects on metabolism parameters***. Significant values (*P* <0.05) shown in bold. 'Temperature', 'sediment' and 'dewatering' show main effects. Other columns indicate interaction effects.

Dependent variable	Temperature	Sediment	Dewatering	Temperature x sediment	Temperature x dewatering	Sediment x dewatering	Warming x sediment x dewatering
ER	0.120	0.830	0.127	0.703	0.745	0.850	0.487
GPP	0.2519	0.6583	**0.0482**	0.5011	0.2827	0.9536	0.3716
ER : GPP	0.793	0.600	0.126	0.476	0.118	0.681	0.514
Benthic respiration(CO_2)	0.222	**<0.001**	0.481	0.958	0.076	0.269	0.437

*See Table B4, Appendix B for three way ANOVA model outputs

Figure 3.15. **Decay coefficient (-*K*) comparison across channel treatments**. Bars show mean (± SE) breakdown of weighed alder leaves in leaf packs. Vertical dashed line separates treatments grouped by sediment. Treatments defined by treatment codes, where C = control, W = warmed, S = sediment and D = dewatered.

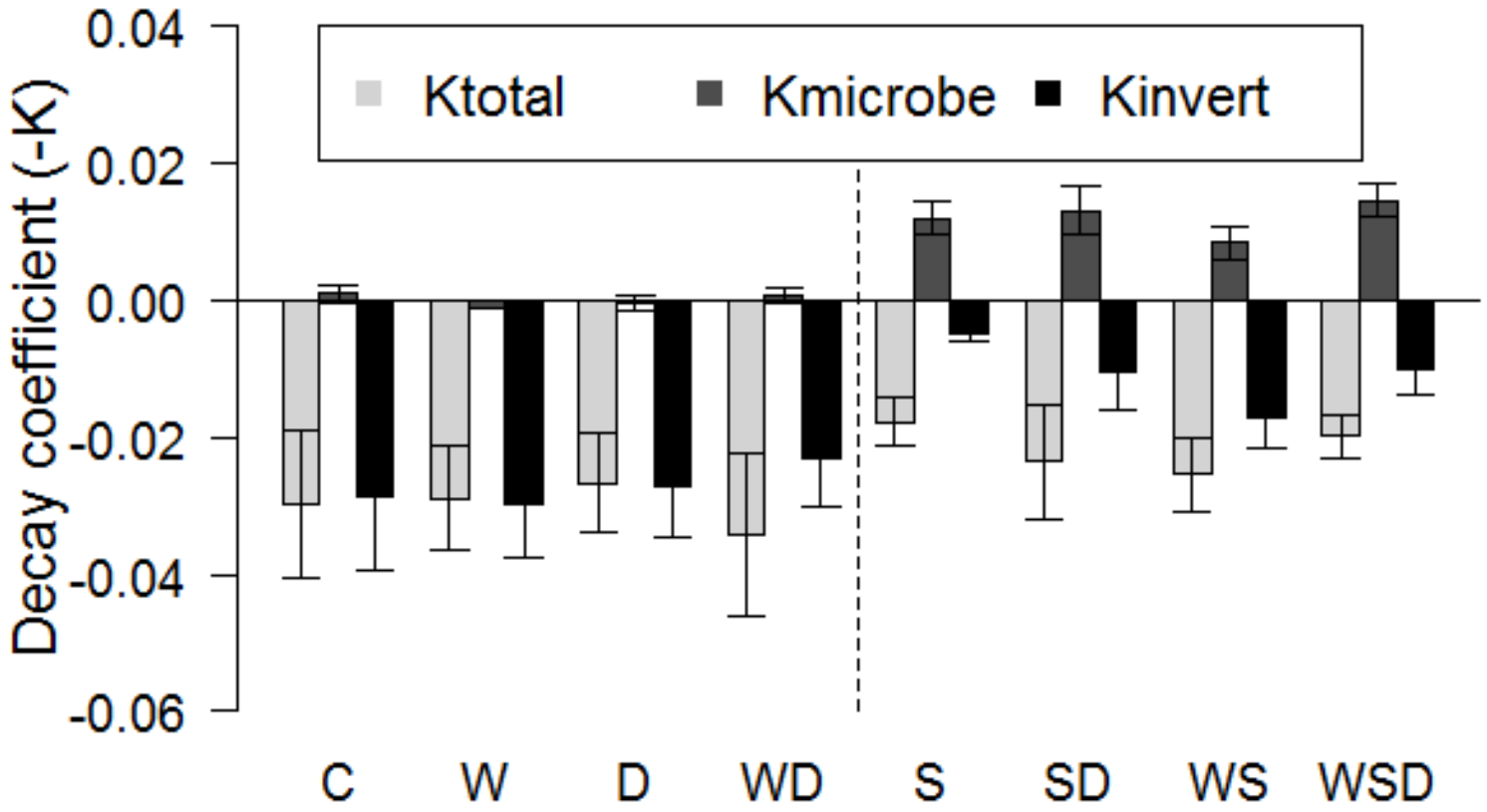


Table 3.4. **Three way ANOVA summary results illustrating stressor effects on leaf litter decomposition parameters***. Significant values (*P* <0.05) shown in bold. 'Temperature', 'sediment' and 'dewatering' show main effects. Other columns indicate interaction effects.

Dependent variable	Temperature	Sediment	Dewatering	Temperature x sediment	Temperature x dewatering	Sediment x dewatering	Warming x sediment x dewatering
K total	0.846	**0.001**	0.593	0.472	0.369	0.713	0.688
K microbe	0.059	**<0.001**	0.217	0.892	0.172	0.290	0.778
K invert	0.653	0.181	0.920	0.888	0.920	0.943	0.409

*See Table B5, Appendix B for three way ANOVA model outputs

Table 3.5. **Mean nutrient concentrations across treatments**. Values = µmol; PO_4^{2-} and NO_3^-. Limits of detection = 0.2 µmol. Treatments defined by codes, where C = control, W = warmed, S = sediment and D = dewatered.

Treatment	PO_4^{2-}	SE	NO_3^-	SE
HEADER	< LOD	-	294.24	-
C	< LOD	-	294.32	6.26
S	< LOD	-	279.72	13.65
D	0.4	0.25	212.27	67.38
W	< LOD	-	280.28	10.34
SD	0.81	0.5	214.20	56.69
WD	0.27	0.16	216.47	62.39
WS	< LOD	-	277.88	8.48
WSD	3.98	1.75	120.59	67.22

3.5 DISCUSSION

It is expected that stressors such as those associated with hydrologic drought will decrease future provisioning of ecosystem services from streams and rivers (Kundzewicz *et al.*, 2008). Knowledge of drought impact causal mechanisms may help mitigate future effects, yet such knowledge remains sparse. This chapter provides some of the first results of individual and combined drought stressor impacts on ecosystem functioning processes. Sedimentation, dewatering and warming were applied to mesocosm channels singly and in combination for the first time. Significant responses were found across all levels of functioning including macroinvertebrate biomass standing stock, macrophyte growth, leaf litter decomposition and metabolism. Stressor main effects were common, whilst interactions were less frequent, similar to findings by others who have investigated multiple stressor impacts on stream functioning (Mustonen *et al*., 2016). Where two stressors interacted, these resulted in negative facilitative effects relative to the individual stressors in 67% of cases. The research suggests drought stressors singly and in combination can suppress stream functioning in some instances, but benefit it in others. In one case, the negative effect of two combined stressors was cancelled by the level of a third stressor. This highlights the complex nature of interactions among drought stressors, and the need to better understand drought stressor interactions in order to ameliorate the predicted negative effects of drought in future when the climate dries.

Macroinvertebrate standing stock

The total biomass of macroinvertebrates was significantly positively affected by an interaction between sediment and warming, whereby warming and sediment

addition combined led to a positive effect on total biomass, relative to the single stressors. This is in contrast to predictions made in Hypothesis 1. It is possible that, whilst only contributing a small biomass relative to other taxa, densities of *r*-selected taxa in WS (warming x sediment; see Chapter Two) resulted in this significant increase in total biomass, as Hypothesis 1 did not account for increases in biomass attributable to *r*-selected taxa. The increase in collectors was largely attributable to *Micropsectra* sp.. This demonstrates the ability of *r*-selected taxa to rapidly occupy stressed environments (MacArthur & Wilson, 1967), as has been found by others studying hydrological drought (Ledger *et al*., 2012).

No statistically significant stressor main effects were detected on total biomass. This is in contrast to a channel dewatering experiment by Walters & Post (2010) who found a decline in aquatic habitat significantly explained a reduction in total macroinvertebrate biomass. However in the current study, a dewatering main effect reduced the biomass of shredders, whilst a warming main effect increased grazer biomass and an interaction between warming and dewatering resulted in a negative effect on collector biomass when warming and dewatering were combined. These findings suggest that differences in response direction among functional feeding groups may compensate one another, in turn offering total macroinvertebrate biomass resistance to drought. Moreover, the response of collector biomass illustrates that combined drought stressors may have more deleterious effects on ecosystem functioning than single drought stressors alone.

When all three stressors were combined, shredder biomass declined by 92% from the control, supporting predictions made in Hypothesis 1, whilst grazers increased by 68%. Main effects on grazer and shredder biomass mirrored taxa populations in

Chapter 2. Thus, biomass effects were principally owing to a decline in the abundance of *G. pulex* and an increase in the abundance of *R*. *balthica* (see Chapter Two). *Gammarus* abundance has been severely reduced by drought in other studies, including Ladle & Bass (1981), whilst at a FFG level, Ledger *et al*. (2013) found dewatering reduced annual shredder biomass by up to 69% and Mariluan *et al*. (2015) found shredder biomass to be 70% greater in a permanent stream compared to an intermittent stream. Findings from this chapter therefore appear to agree with other studies that shredders may be particularly susceptible to drought. This decline may be the result of a combination of reduced secondary production (Ledger *et al*., 2011) and abundance (Ledger *et al*., 2012). Additionally, diminishing food resources might have partly contributed to the decline in shredders (Lake, 2003). However, a reduction in resources was not considered a flaw of the experimental design, as resources were still observed at the end of the experiment. Acuña *et al*. (2005) also demonstrate aquatic biomass follows density patterns, suggesting differences from modified production rates may be less significant than those attributable to altered abundance, and supporting the observation of a strong link between biomass and abundance in this research.

Primary Production

Ranunculus pseudofluitans growth was negatively affected to a greater extent than *B. erecta*, highlighting the greater sensitivity of submerged macrophytes to drought stressors and supporting predictions made in Hypothesis 2. These findings support the notion that emergent species may have a competitive advantage during drought (Wright & Berrie, 1987; Westwood *et al*., 2006; Boulton, 2003). The findings suggest that warming, dewatering and sedimentation may all decrease submerged aquatic

macrophyte growth, also evidenced by Lacoul & Freedman (2006). This may have further implications on aquatic animals by reducing habitat availability, though the dead organic matter may serve as a source of damp refugia in the event of total streambed desiccation. Warming had a significant negative main effect upon *R. pseudofluitans* growth rate but a positive effect upon that of *B. erecta*, suggesting that submerged macrophytes may have a narrower thermal optimum window than emergent species, though further work investigating macrophyte optimum growth temperatures is required to confirm this speculation. Owing to the high specific heat capacity of water, submerged plants occupy a narrow thermal range relative to the more variable atmospheric temperature range that emergent plant species occupy. Emergent taxa have been shown to be more tolerant of desiccation during drying than submerged taxa (Boulton, 2003) and thus it is possible that thermal optimum, and tolerance, of macrophytes correlates with desiccation resistance.

All drought stressors produced a significant three way interaction on the growth of *B. erecta*. This can be explained as the interaction effect of two stressors being determined by the level of a third stressor. In this specific case, warming significantly increased *B. erecta* growth rate, but this was determined by the level of sediment, i.e. when warming was combined with sediment (WS), *B. erecta* growth rate was not significantly different from the control (neutral [inhibitive] interactive effect). However, the interaction of sediment with warming was dependent on the level of dewatering. Whilst warming and sediment combined (WS) were not significantly greater than the control mean, when combined with dewatering (WSD), this neutral interactive effect of sediment on warming was lost, and the WSD mean was significantly greater than the control, and similar to W. The effect of all three

stressors combined was positive and resulted in increased production compared to the effect of sediment and warming combined.

Stressors had main effects on the photosynthetic capacity of the two macrophyte species, but whereas effects were mostly positive for *B. erecta* growth rate and negative for *R. pseudofluitans*, effects on chlorophyll concentration were negative for both *B. erecta* (warming main effect and sediment main effect) and *R. pseudofluitans* (dewatering main effect) contrasting predictions made in Hypothesis 2. Despite dewatering elevating *R. pseudofluitans* photosynthetic capacity, the main effect of dewatering reduced production, and suggests that photosynthetic capacity as an indicator of macrophyte health may not accurately portray an individual's ability to grow. It is thought that *R. pseudofluitans* underwent a shift from standard growth to production of woody tissue and carotenoids to adapt to the amphibious environment and provide protection from elevated insolation (Demmig-Adams & Adams, 1996). Reduced biomass of *Ranunculus* spp. during drought was also observed by Wright & Berrie (1987) owing to decomposition of plant biomass, and probably too due to reduced production. An increase in *B. erecta* growth as observed, may result in increased terrestrial primary production, shifting energy flow pathways from aquatic to terrestrial during drought (O'Callaghan *et al*., in prep). Dewatering increased macrophyte growth and chlorophyll concentration in a study by Bucak *et al*. (2012) and emergent leaf forms had greater chlorophyll concentrations relative to submerged forms in studies by Nielsen & Sand-jensen (1997) and Pedersen & Sand-jensen (1992), highlighting the potential for primary production to respond positively to drought.

An interesting observation from this experiment was the noticeable change in *R. pseudofluitans* morphology within dewatered channels. The divided leaves of the terrestrialised individuals consisted of shorter leaflets, and the plant became more rigid. Such responses are believed to be an attempt to reduce self-shading, and to aid plant support (Germ & Gaberscik, 2003). These plants were also much smaller, probably in an attempt to reduce water loss through the newly formed stomata of the terrestrialised leaves (Cook, 1969). The mechanisms underpinning these morphological adaptations explain the reduced rates of production above, and highlight that macrophyte winners and losers result from drought – with submergent taxa losing in order to adapt and survive, whilst emergent taxa go largely unperturbed.

Metabolism

ER and GPP were similar in regards to magnitude of effect across treatments. However, stressors had no significant effects on ER, and differences across treatments were not detected. On the other hand, a main effect of dewatering was found on GPP, with GPP elevated in dewatered channels. Although similar patters were found for ER and GPP, it is likely that non-significant effects on ER were attributable to greater variability within treatments.

Generally all treatments elicited heterotrophy with the exception of the three combined stressor treatment (WSD). Heterotrophy is common in natural stream systems (Cole & Caraco, 2001) owing to terrestrial allochthonous subsidies. Whilst warming increased both GPP and ER, this increase was not always proportionate, resulting in discrepancies between the two (i.e. differences in NEP). Yvon-Durocher

et al. (2010) concluded that the increased discrepancy between ER and GPP with warming (i.e. warming increased the rate of ER disproportionately greater than GPP) resulted in a loss of carbon sequestration. In the present study, the discrepancy between ER and GPP was greatly increased with warming (increased ER : GPP), resulting in a greater degree of heterotrophy ER > GPP, and a 153% reduction in carbon sequestration, supporting Hypothesis 3. Findings from the present study would thus agree with Yvon-Durocher *et al*. (2010) – that future warming may reduce stream ecosystem carbon sequestration. This has the potential to limit secondary production and increase greenhouse gas emissions from streams, leading to positive feedback loops, in turn warming streams further. However, this observation may in fact be only a short term non-steady-state response (i.e. metabolic equilibrium may eventually be reached) as plant respiration is ultimately limited by carbohydrates fixed through plant photosynthesis (Allen *et al*., 2005; Dewar *et al*., 1999).

When all stressors were combined (WSD), ER was disproportionately lower than GPP and the system became net autotrophic, opposing predictions made in Hypothesis 3. Temperature may partly explain this, with Shen *et al*. (2015) illustrating that heterotrophic systems become autotrophic during summer months, though increasing concentration of limiting nutrients are believed to play an important role; limiting nutrients (i.e. P) can concentrate in dewatered channel sections (Acuña *et al*., 2005; Dollar *et al*., 2003) due to phosphate release from the sediment (van Vliet & Zwolsman, 2008), which is exacerbated during oxygen depletion (House & Denison, 2000; Wetzel, 2001) and reduced dilution capacity (Mosley, 2015). Phosphate concentrations were below the LOD in abstracted

groundwater and thus comparable to concentrations measured in other studies (e.g. ~0.2 µm l^{-1}; Bryan *et al*., 2015; Jarvie *et al*., 2005; Pretty *et al*., 2006), maintained at low concentrations due to co-precipitation of P with C_aCO_3 (Lapworth *et al*., 2011). In the mesocosms, phosphate in the added substrate may too have been attached to iron hydroxide minerals in the sediment (House, 2003). However, dewatering in the mesocosms lead to the accumulation of detectable P, and the concentration of P was greatly increased, relative to the groundwater source, when all three stressors were combined (WSD). Concentrations in the WSD treatment had the potential to greatly increase algal growth (Mainstone & Parr, 2002) which may have elevated photosynthesis (House, 2003) and lead to net autotrophy as demonstrated by Peterson *et al*. (1985). Whilst algal biomass was not quantified in this experiment, and no obvious algal growth increase in stressed channels was observed, it remains possible that chlorophyll concentrations increased in response to elevated P (Peterson *et al*., 1985). Conversely, nitrate showed the opposite response to phosphate, and whilst elevated photosynthesis may in part explain this (i.e. increased uptake and assimilation of nitrate by algae [van Vliet & Zwolsman, 2008] and *Ranunculus* spp. [Prior & Johnes, 2002]), it is more likely that under conditions which increase the rate of phosphate release from the sediment (i.e. anaerobic), nitrate is used as an alternative electron acceptor and subsequently reduced (i.e. denitrification; Rivett *et al*., 2008) supporting the proposed theory of P release from the sediment. Although anaerobic conditions that could lead to exacerbated P release and denitrification (< 1-2 mg l^{-1}; Rivett *et al*. 2008) were not evidenced from the diel DO curves (DO always above ~2 mg l^{-1}), it should be noted that DO was only measured above the benthic surface. Anaerobic conditions can often occur

beneath the benthic surface in sediments as shallow as 5 cm (Malard & Hervant, 1999) and thus may have occurred within the mesocosms during the duration of the experiment. Further work could measure DO both above the sediment surface and below 5 cm depth to determine whether anaerobic conditions occur in drought applied mesocosms. Overall, an interesting story is illustrated based on interactions between applied stressors, dissolved oxygen, and nutrients, which suggest alterations to the fate of carbon during drought. These snapshot findings allow a comparison of channels within and across treatments, but it must be stressed that a greater temporal resolution is needed to derive conclusions with increased confidence, as the sampling regime did not provide a representative picture of channel water chemistry across both space and time. As observed net autotrophy in the WSD treatment was statistically non-significant, this finding should be considered carefully and further investigation is required before sound conclusions can be drawn. A decline in allochthonous leaf litter processing attributable to a severe reduction in shredder biomass may change the system to autotrophy dependence (Humphries & Baldwin 2003) and further research is needed to confirm whether this occurs.

Sediment invoked a main effect on benthic respiration in the separate respiration experiment, with sediment addition increasing microbial respiration, supporting predictions in Hypothesis 4. Sedimentation, which may increase during drought, has also been shown in other contexts to increase benthic respiration in natural stream systems (Shelly *et al*., 2015). Not correcting for OM, sediment alone increased respiration by 64% relative to the control (coarse gravel) in the current respiration experiment. An increase in the surface area upon which respiring organisms can

attach, along with an increase in the addition of OM (+20.13% ± 2.53) are both likely to have played major roles in increasing the rate of benthic respiration. As drought (specifically sedimentation) increased benthic respiration, H_4 can be accepted.

Sand-Jensen *et al*. (2007) predict that an increase in water temperatures of 2.2-4.5 °C in future will increase bacterial respiration by 26-63%. In the present study, sediment (in the laboratory experiment) was found to have a greater impact on microbial activity than warming. However this was expected, as all vials were acclimated to the same temperature of 15 °C. Whilst W and WD mean respiration values were +4.5-18% greater than the control, warming coupled with sedimentation (WS, WSD) resulted in greater respiration values of ~50 and 55% respectively, relative to the control. In order to determine whether sediment or warming was the most pervasive stressor during drought, further work should investigate benthic respiration of sediment samples *in-situ*, in order to incorporate combined thermal differences between treatments.

Organic Matter Decomposition

The decomposition of OM is an important functional metric because it can provide a surrogate of allochthonous basal resource incorporation into the aquatic food web (Tank *et al*., 2010). Sediment had a main effect on both Ktotal and Kmicrobe, significantly decreasing decay coefficient rates. Fine leaf pack biomass surprisingly increased in channels supplied with sediment following the termination of the experiment, suggesting rapid periphyton accrual (enhanced as a result of increased OM and nutrients) exceeded the rate of leaf litter breakdown. Moreover, it is thought

that small macroinvertebrates such as *Micropsectra* sp. were able to access fine leaf packs, grow, and become trapped, and these individuals may have contributed to the overall biomass following the experiment. Whilst the size of the fine mesh packs have been used in other studies (e.g. Boyero *et al*., 2011; Graca, 2001; Riipinen *et al*., 2010), *Micropsectra* spp. have been found capable of entering 500 µm mesh leaf packs (Schlief & Mutz, 2009). As a result, it can be concluded that sediment significantly reduced the rate of leaf litter decay, but that the precise rate of decay must be interpreted with caution owing to the increased mass of fine leaf packs following the termination of the experiment.

Previous studies have demonstrated that a reduction in shredder FFG biomass can result in a decrease in OM breakdown (Chadwick & Huryn, 2005; Domingos *et al*., 2014; Martínez *et al*., 2013). Furthermore, stressors used within this study have been shown to affect OM decomposition elsewhere (e.g. Piggott *et al*., 2012; Correa-Araneda *et al*., 2015; Friberg *et al*., 2009; Perkins *et al*., 2010; Dang *et al*., 2009; Magoulick, 2014). However, there appeared to be no relationship between shredder biomass and OM decomposition (i.e. a 50% reduction in shredder biomass with dewatering did not significantly reduce the decomposition of alder leaves). This could be due to shredder reductions unknowingly occurring near to the termination of the experiment, before which shredder biomass (and therefore macroinvertebrate induced decomposition) may have been similar among treatments. In order to test this hypothesis, and due to a lack of observational or quantifiable record of shredder abundance throughout the experiment, further work would need to investigate leaf litter decomposition and shredder biomass at periodic intervals, as opposed to simply following the termination of the experiment only, as was the case in this

study. This would allow changes in the biomass of shredders and leaf litter decomposition to be correlated over time, to determine whether losses or gains in shredder biomass influenced decomposition rates. Whilst elevated temperatures compensated for a reduction in leaf litter decomposition by macroinvertebrate shredders in a study by Mariluan *et al*. (2015) by enhancing microbial breakdown, there was no evidence of this in the current study, with macroinvertebrate feeding contributing to the majority of leaf litter breakdown among treatments, with contributions from microbial activity being negligible. However these findings suggest that decomposition rates may be context dependent and further research is needed in order to draw overall conclusions. A higher frequency of decomposition sampling may also increase the likelihood of depicting causal mechanisms.

Gessner & Chauvet (2002) proposed that OM decay coefficients between 0.1-0.3 are indicative of good ecosystem health, whereas values above and below suggest negative effects upon overall health. Typically, decay coefficient values in this study were between 0.1-0.3 (with the exception of WD where decay coefficients were marginally greater). However, other measured responses did not appear most negatively affected in WD channels, suggesting that rates of decomposition in the mesocosm channels did not correlate with overall health.

Niyogi *et al*. (2003) found respiration correlated significantly with leaf litter decomposition but, owing to positive decay coefficients in the current study, it was not possible to identify a relationship between decomposition and microbial respiration. Findings from this study also illustrate the importance of recording functional parameters across a range of environmental conditions and geographical

localities (Bruesewitz *et al*., 2013) as findings did not always correspond to previous findings from other studies.

3.6 CONCLUSION

This study provides some of the first research to investigate causal mechanisms of specific drought stressors on functional processes and provides evidence that stressors can produce unexpected ecological effects through complex interactions in addition to main effects. Changes to the biomass of functional feeding groups could reduce the importance of allochthonous resources and intensify grazing pressure; disproportionate changes to macrophyte growth may alter energy flow pathways from aquatic to terrestrial, whilst elevated rates of GPP and benthic respiration may alter carbon availability and storage. The challenge now is to conduct similar experiments at larger and more natural spatial scales, as well as longer temporal scales, to determine drought stressor effects over supra-seasonal timescales and to extrapolate findings to natural settings more easily. Moreover, manipulations incorporating thresholds earlier and later in the drought sequence (i.e. cessation of flow from lotic to lentic, and complete dewatering leading to total water loss, respectively) are needed in order to incorporate crucial ecological thresholds that were excluded from the current study.

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CHAPTER FOUR

Sedimentation intensifies predator-prey interactions in rivers: evidence from a comparative functional response experiment

4.1 ABSTRACT

Sediment deposition in river networks has become increasingly problematic in recent years due to the intensification of land use and agricultural practices, poor water management and modified stream morphology. The direct effects of sedimentation on stream ecology have been widely studied, yet little remains known regarding indirect biotic effects mediated through the food web. This chapter examines the potential for sediment addition to increase the strength of the interaction between a benthic predator - the bullhead (*Cottus gobio*) – and one of their common benthic macroinvertebrate prey – the freshwater shrimp (*Gammarus pulex*). Specifically, bullhead feeding rates were measured in a functional response feeding experiment with two substrate treatments (sediment vs. non sediment). Sedimentation greatly increased the efficiency of the predator (increasing attack rate), in turn increasing proportional prey consumption. Proportional consumption was best explained by a logistic regression model incorporating an interaction between substrate and initial prey density. This interaction was explained by greater substrate effects at lower prey densities, but no substrate effects at larger prey densities owing to saturation. This study demonstrates how strengthened biotic interactions during sedimentation events may exert a dominant influence over the fate of remnant prey populations following sedimentation, increasing the likelihood of local prey extinctions and in turn reducing stream resilience. Moreover the strength of top-down control is demonstrated to be greatly affected by the availability of prey. Local prey extinction is most likely where low prey density is coupled with sedimentation.

4.2 INTRODUCTION

Streams and rivers now face an ever increasing threat from stressors including pollution, invasive species, and sedimentation (Strayer & Dudgeon, 2010; Dudgeon *et al*., 2006; Ormerod *et al*., 2010). Sedimentation is a natural process (Wood & Armitage, 1997), but anthropogenic activities increase loading from the surrounding catchment (Allan, 2004; Walling & Amos, 1999). Agriculture, construction and industry all contribute significant quantities of sediment to running waters (Harding *et al.*, 1999; Ryan, 1991). In recent years, sedimentation has also increased as a result of river regulation and modification (Jones *et al*., 2015) and logging of forests for timber (Kreutzweiser *et al*., 2009; Moring, 1982) whilst climate change may increase land-based source contributions through processes such as desertification (Chen & Lian, 2016). It is widely recognised that sediment can have major effects on aquatic biota and sedimentation events have been identified as an important stressor in streams and rivers (Lemly, 1982; Jones *et al*. 2012b; Kochersberger *et al*., 2012).

Sedimentation can alter benthic community composition (Wood & Armitage, 1997), typically reducing species richness and total abundance (Bo *et al*., 2007; Buendia *et al*., 2013; Connolly & Pearson, 2007; Couceiro *et al*., 2011; Larsen *et al*., 2011; Ramezani *et al*., 2014). Biotic indices such as the percentage of Ephemeroptera, Plecoptera and Trichoptera (%EPT) have also been shown to strongly correspond to sediment metrics (Sutherland *et al*., 2012). Fine sediment deposition can increase the prevalence of *r*-selected taxa (Nuttall & Bielby, 1973), particularly sediment tolerant taxa such as some Chironomidae and Oligochaeta species (Ciesielka & Bailey, 2001; Downes *et al*., 2006), whereas more sensitive taxa such

as many filterers (e.g. mussels) are eliminated altogether (Geist & Auerswald, 2007). These changes to the community composition are the result of either direct (abiotic) or indirect (biotic) mechanisms (Jones *et al*., 2012a).

Direct effects of sedimentation include the clogging of organism respiratory structures (e.g. gills) by settling particles (Lemly, 1982) and in extreme cases of deposition, complete burial of biota may occur, smothering taxa and preventing them reaching the surface (Chandrasekara & Frid, 1998; Wood *et al*., 2005). Burial of eggs may reduce hatching success of macroinvertebrates (Kefford *et al*., 2010) and fish (Moring, 1982). Clogging of the substrata can form an impermeable layer, preventing diffusion of oxygen and producing hypoxic conditions (Jones *et al*., 2012a), in turn killing taxa sensitive to low dissolved oxygen (Verberk & Bilton, 2013). Furthermore, contaminants may adsorb to sediment particles, resulting in water quality deterioration (Burton & Allen, 1991). Physical barriers produced by deposited sediment may also impede the movements of taxa on the streambed (Mathers *et al*., 2014).

Sedimentation may also arise in a number of *indirect* effects, mediated through the benthic food web. Ecological responses to biota following sedimentation constitute a secondary response, yet the implications for prey populations may be more significant than primary abiotic impacts. For example, resources may become buried (Jones *et al*., 2012b), triggering bottom-up regulation of the biotic community. Disproportionate affects among key ecological groups (Couceiro *et al*., 2011) may modify functional processes, which subsequently ripple through the food web as energy flow pathways between resources and top predators change. Interstitial spaces between substrate particles, which ordinarily provide predator avoidance

refugia for important stream taxa such as *Gammarus pulex* (McGrath *et al*., 2007), may become clogged, altering prey vulnerability to predators. Infilling of entire mesohabitat patches (Doeg & Koehn, 1994) may reduce habitat availability (Burdon *et al*., 2013) and alter the encounter rate between prey and predators, intensifying biotic interactions (Martin *et al*., 2012; Bond *et al*., 2008), exacerbating prey consumption (Clark *et al*., 2013) and reducing prey population density (Peckarsky, 1985). Despite sedimentation having been studied for the last 60 years (Extence *et al*., 2013), quantifying indirect biotic effects is more challenging and understanding still lags behind that of direct abiotic effects. Predator-prey functional response (FR) experiments may provide a useful and novel tool to investigate indirect impacts of global change (O'Gorman, 2014), including sedimentation.

In lowland rivers, low flow events (e.g. hydrologic drought) can increase sedimentation (Wood & Armitage, 1999). Natural low flow variability may also be exacerbated by anthropogenic pressures including water withdrawals for public supply (Lake, 2011), further increasing the likelihood of sedimentation. Severe flow reduction can fragment aquatic habitat into a series of isolated pools (Boulton, 1990; Lake, 2003) which may result in *sediment x dewatering* compound stress. Nonetheless these pools offer important refugia allowing taxa to escape drying riffles and avoid desiccation (Avery-Gomm *et al*., 2014; Covich *et al*., 2003; Dewson *et al*., 2007). If able to mobilise in response to drying ques, animals from numerous trophic levels may aggregate in these pools, confining predators and their prey into close proximity, which may intensify their interactions (Dollar *et al*., 2003). Intense predation within pools constitutes an indirect effect of drought that could lead to local suppression or extirpation of prey species, but empirical studies quantifying

biotic interactions remains scarce. The return of faster flows typically flushes sediment from the channel bed, quickly restoring streambed morphology (Schälchli, 1992). However local prey extinction as a result of intensified top-down control could hamper rapid ecological restoration, which could even reduce the capacity of streams and rivers to provide ecosystem services (Schlief & Mutz, 2009).

A number of different approaches exist to determine the relationship between sedimentation and biotic interactions including empirical surveys, models, and experiments. Whilst survey approaches may offer the highest degree of naturalness, they too can often be confounded by extraneous confounding variables (Harris *et al*., 2007). Modelling approaches such as Bayesian Belief Networks are an alternative approach to determining sediment impacts that are based on expert opinion and subsequent validation from survey data, but are constrained by the challenge of finding suitable environmental conditions to validate models (Allan *et al*., 2012). Experimental approaches using outdoor mesocosms allow sediment to be manipulated whilst all other environmental parameters are held constant (Stewart *et al*., 2013), enabling causal mechanisms between sediment and biotic interactions to be determined.

A functional response describes the relationship between prey density and consumption by predators (Holling, 1959) and can provide useful outputs (Juliano, 2001) including the attack rate (predator efficiency; McPhee *et al*., 2012) and handling time (prey processing; Kislalioglu & Gibson, 1976) allowing predation pressure with and without the addition of fine material to be quantified. It is possible that sedimentation, through habitat simplification and a numerical reduction in interstitial spaces, may increase both the proportion of prey consumed (Crowder &

Cooper, 1982), and the predator attack rate (Manatunge *et al*., 2000). Whilst it is normally difficult to separate the effects of reduced flow and sedimentation, here a mesocosm approach allowed sedimentation to be varied under low flow conditions using a predator-prey FR experiment to test two hypotheses: H_1 proposed that sedimentation would increase the predator attack rate, due to increased predatory efficiency in simplified habitat mesocosms. H_2 proposed that sedimentation would significantly increase proportional prey consumption due to a loss of interstitial space and increased predator-prey encounter.

4.2.1 Taxa selection

Bullhead (*Cottus gobio*), a benthic predator, and the 'freshwater shrimp' (*Gammarus pulex*), an amphipod prey, were selected as model organisms for the feeding experiment. Both *C. gobio* and *G. pulex* are often extremely common species in lowland streams of the U.K. (Harrison *et al*., 2005) and can both exert powerful effects upon stream community composition (Woodward *et al*., 2008). *Cottus gobio* have been kept successfully under experimental conditions and have been shown to feed normally in artificial habitats (Elliott & Elliott, 1995). Additionally, *G. pulex* often constitute a large proportion of fish diets (Hughes and Croy, 1993; Macneil *et al*., 1997) including *C. gobio* (Davey *et al*., 2006), and have previously been used as a model organism in other sediment context experiments (e.g. Vadher *et al*., 2015).

4.3 METHODOLOGY

A mesocosm experiment was conducted during September 2013 to test for the effect of sedimentation on the functional response of *C. gobio* feeding upon *G. pulex*.

Feeding trials were conducted in mesocosms (Fig. 4.1) sited outdoors adjacent to a chalk stream at a watercress farm in Southern England (51°6'4"N, 1°11'13"W). Mesocosms were plastic aquaria (length = 43 cm, width = 33 cm, height = 25 cm, volume = 35 litre) fed by cool (10.3 °C), well-oxygenated (10.58 mg l^{-1}) groundwater from a borehole (2.5 l min^{-1} per aquarium). The substratum in mesocosms consisted of pebbles and gravel (5 cm depth; interstitial volume = 49 %) in controls, and sand (5 cm depth; interstitial volume = 38 %) in the sediment addition treatment. Substrata cumulative percentage frequencies for each treatment are illustrated in Fig. 4.2 (see Table C1 and Fig. C1, Appendix C for additional information). Sand was used over natural sediment, because it provided particle size consistency among mesocosms thus improving replicability, and provided more efficient substrate handling and prey retrieval, in turn reducing time in between experimental runs. It was deemed that these advantages outweighed any cons of using sand, e.g. lack of realism. Each mesocosm contained a shelter (20 cm section of 110 mm pipe cute lengthways) to minimise stress and mimic the natural habitat of the fish (i.e. dark environments beneath stones). Bullhead (age = 2 – 3 years) of a standard size (mean length 7.1 ± 0.1 cm; mean biomass 5.0 ± 0.3 g) were collected from the adjacent Candover Brook using a hand net and transferred to a storage tank supplied with groundwater, before introduction to the mesocosms. Amphipod prey were collected from drainage channels within the watercress farm, and were

individually selected based on their size (~5 mm), which was visually assessed in the field. Fish were reused following 24 hours starvation between trials, with trials randomised throughout time, and then released.

In each of the two substrate treatments (cobble control vs. sand sediment treatment; Figs. 4.1-4.2), individual fish were supplied with *G. pulex* at 18 different prey densities (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, 75, 100, 150, 200, 500 and 1000 individuals per mesocosm) with eight replicates of each density (i.e. two substrate treatments x 18 prey densities x eight replicates = 288 trials in total). This range of prey gave densities of 8.3 – 10790 animals m^{-2}, encompassing natural densities found within a nearby lowland chalk stream (80 – 2250 m^2; Williams *et al*., unpublished data) and other cited densities (Harrison *et al*., 2005; Wright *et al*., 2004; Wright & Symes, 1999; Wright, 1992).

The number and proportion of prey remaining in each mesocosm was determined after 24 hours exposure to the predator. An additional fishless treatment at each prey density determined proportional mortality in the absence of the predator.

Figure 4.1. **Photograph of mesocosms**. Taken shortly after construction (August 2013). Showing (a) cobble control and (b) sand sediment treatment

(a)

(b)

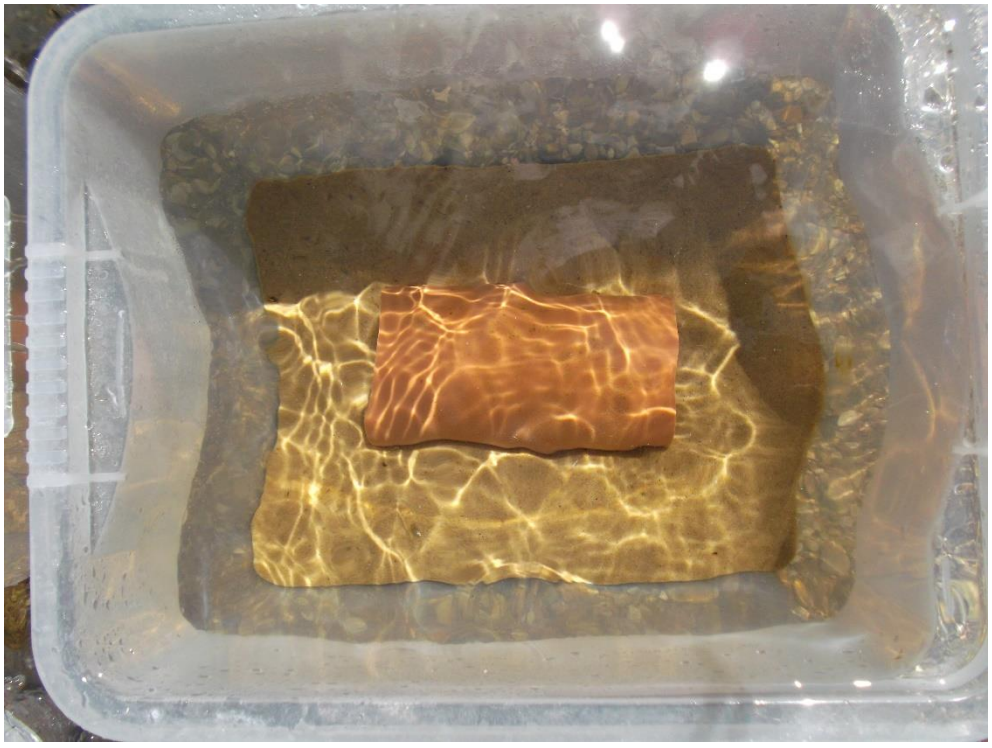
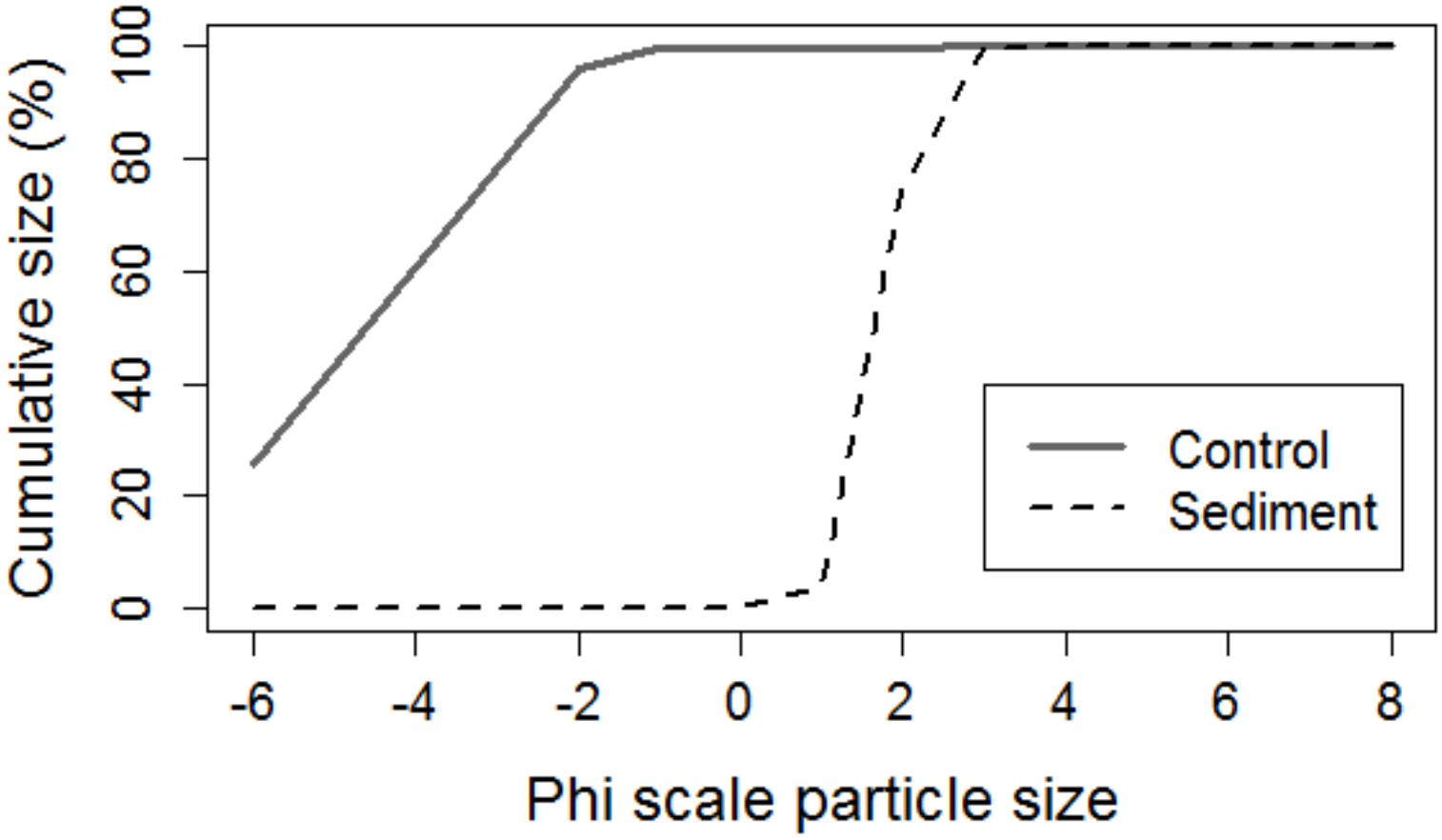


Figure 4.2. **Physical treatment characterisation of the mesocosms**. Solid line (control) and dashed line (sand sediment treatment) illustrates the cumulative percentage frequency of substratum particle size distribution. N.B Phi scale units.



4.3.1 Data analysis

All data visualisation and statistical analyses were conducted using R, version 3.2.0 (R Core Development Team, 2015).

Initial curve visualisation was conducting using the R package “frair” (Pritchard, 2014), which uses nonlinear boot strapping to produce FR curves with 95% CI shaded polygons.

A type II FR model (Eq. 1) was fitted to the experimental data using the “emdbook” package (Bolker, 2015), which uses a nonlinear least squares (‘nls’) method, after Rall *et al*. (2011). The equation (Rogers Random Predator [RRP; Rogers, 1972]) uses the feeding experiment data (initial prey density vs. number of prey eaten) and allows for prey depletion over time (i.e. no replacement). Type II was selected as the experiment consisted of a predatory fish and single prey species (Murdoch & Bence, 1987). Type II responses elicit a curve whereby prey consumption increases at a decelerating rate and eventually plateaus (Holling, 1959), and are typically destabilising as total proportional prey population consumption can occur.

$$N_e = N_i - W\left(ahN_i e^{-a(PT - hNi)} / ah\right) \qquad \text{(Eq. 1)}$$

Here, *Ne* is the number of prey eaten, *Ni* is the initial prey density, *W* is the Lambert W function (see Haddaway *et al*., 2012), *P* is the predator density and *T* is the experiment duration. *h* is the handling time intercept and *a* is the attack rate intercept, which was used in order to test H_1.

Proportional prey consumption were plotted against initial prey density (*Ni*), after Haddaway *et al*. (2012). Logistic regression was conducted in R using the Generalised Linear Model function 'glm' to determine how proportional consumption varied as a function of both substrate and *Ni*. The family argument was specified as 'binomial', which informed the model that the data did not meet assumptions of normal distribution. Owing to the non-linearity of the data, a polynomial argument was included in the models. Four separate models were run, each investigating different predictor variables and combinations (density only, substrate only, density and substrate, and density, substrate and their interaction) on proportional prey consumption. Following this, McFadden's R^2 (McFadden, 1974) was calculated using the log likelihood values of each fitted model, along with a null model (replaces covariates with 'intercept only'), i.e. glm(y ~ 1). McFadden's R^2, along with Akaike Information Criterion (AIC), were used to determine which model provided the best overall fit. If a significant interaction between density and substrate was detected, further exploration was performed by visualising model three-dimensional response surfaces after Lange *et al*. (2016) using the package 'visreg' and the function 'visreg2d' to determine how both substrate and density interacted to affect the regression model response.

4.4 RESULTS

Gammarus pulex survival was always >98% in the absence of *C. gobio*, and as such experimental deaths, following Alexander *et al*. (2015), can be confidently attributed to predation by *C. gobio*. *Cottus gobio* were effective predators of *G. pulex*, with a mean consumption at maximum prey density of 72.14 ± 5.30 *G. pulex* individuals within 24 hours, until satiated.

Functional response model analysis indicated that *C. gobio* attack rate increased 11 fold with sedimentation (a = 5.939 ± 0.390, sediment treatment vs. 0.550 ± 0.026, control treatment). Sediment addition increased handling time by 7% (0.013 ± 0.001 and 0.014 ± <0.001 for control substrate and sediment treatment, respectively). The plotted curves (Fig. 4.3), in agreement with the model output parameter values, showed a steeper ascending gradient for the sediment addition curve relative to that of the control substrate treatment, indicative of increased predator efficiency.

Cottus gobio consumed a greater proportion of *G. pulex* individuals within the sediment treatment relative to the control and consumed proportionally fewer individuals with increasing prey density (indicative of type II; Haddaway *et al*., 2012). The effect which sedimentation had on proportional consumption at lower prey densities was absent at high prey densities, illustrating saturation in both treatments. These effects are illustrated visually in Fig. 4.4 (see Fig. C2, Appendix C for numerical abundance consumption).

Logistic regression analysis revealed that the model incorporating both predictors (substrate and density) and their interaction provided the best fit to the proportional consumption data (AIC = 207.59, McFadden's R^2 = 0.52; Table 4.1). Sediment increased proportional consumption, whereas increasing density reduced it (logistic regression; positive and negative coefficient values, respectively; Table 4.1). The interaction was explained by a greater substrate effect at low prey densities (proportionally fewer prey individuals consumed in control) than high prey densities (proportional consumption similar across both substrate treatments) (Fig. 4.5).

In all models, residual deviance was much lower than null deviance (predicted by a model that only includes intercept), illustrating the importance of predictor variables in explaining proportional prey consumption.

Figure 4.3. **Bullhead type II functional response curves**. Polygons represent relationship between *Ni* and *Ne* (bootstrapped consumption data, 95% CI) following 24 hours of feeding by *C. gobio on* randomly allocated densities of *G. pulex*.

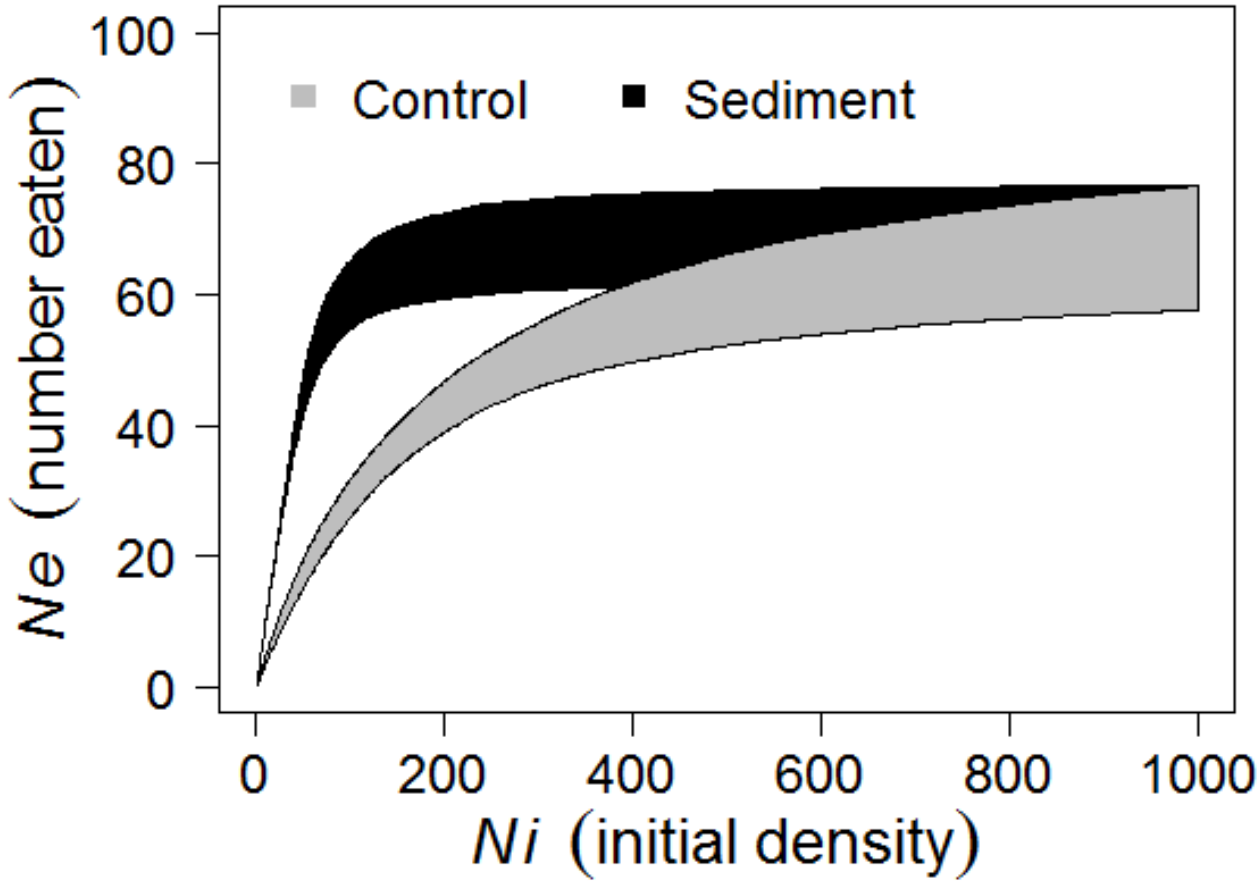


Figure 4.4. **Proportional consumption of *G. pulex* following 24 hours feeding by bullhead.** Bars represent mean (±1SE) consumption of *G. pulex* by bullhead as a percentage of initial prey density. N.B satiation at ~75 individuals.

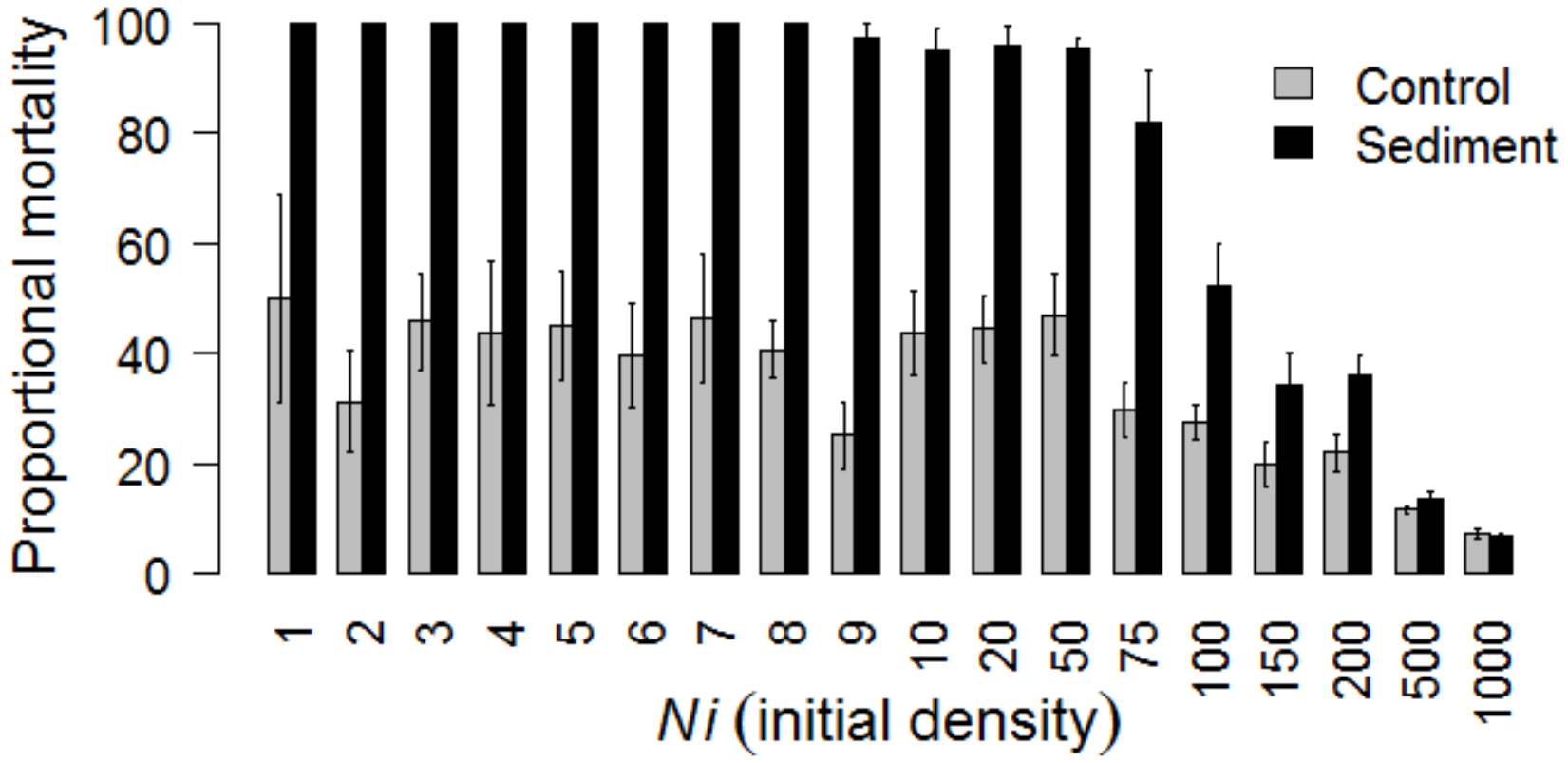
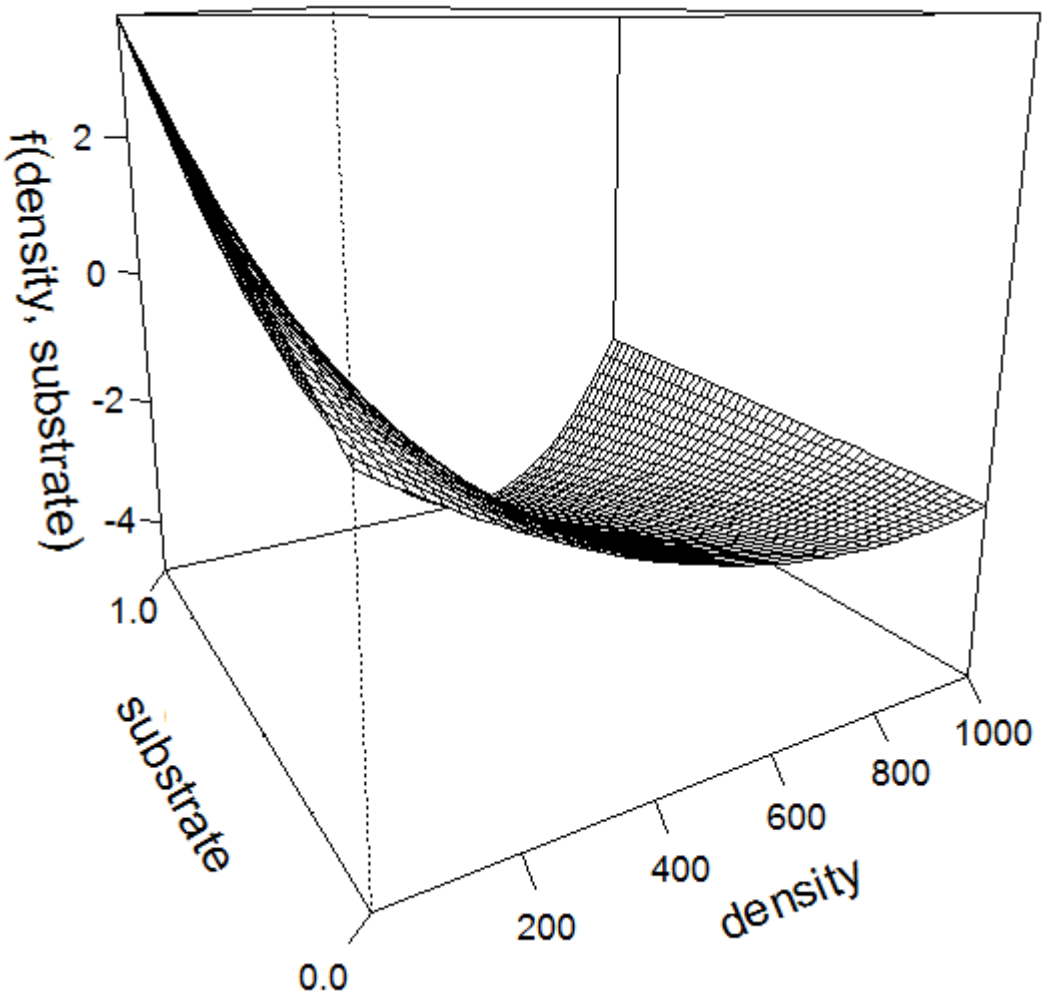


Table 4.1. **Logistic regression summary table.** Illustrating maximum likelihood estimate of proportional prey consumption as a function of initial prey density. Estimate = coefficient of predictors (substrate and density). AIC and R^2 used to determine model goodness of fit.

Model	Predictor	Estimate	Std. Error	z value	*P*	AIC	McFaddan's R^2
1	Substrate	1.9198	0.2674	7.181	<0.001	327.06	0.19
	Null deviance: 207.18 on 287 df; Residual deviance: 149.08 on 286 df						
2	Density	-1.01063	0.02368	-4.488	<0.001	333.77	0.21
	Null deviance: 207.18 on 287 df; Residual deviance: 150.64 on 285 df						
3	Substrate Density	2.619 -0.01473	0.355 0.02937	7.377 -5.014	<0.001 <0.001	220.37	0.48
	Null deviance: 207.177 on 287 df; Residual deviance: 76.135 on 284 df						
4	Substrate Density Substrate:Density	3.897 -0.005711 -0.02091	0.5933 0.003338 0.006337	6.569 -1.711 -3.300	<0.001 0.087 <0.001	207.59	0.52
	Null deviance: 207.18 on 287 df; Residual deviance: 61.16 on 282 df						

Figure 4.5. **Logistic regression model 4: partial residual visualisation.** Perspective plot showing the regression surface, illustrating 1) greater proportional consumption at lower prey densities, and 2) the greater effect of substrate type at lower prey densities in comparison to larger prey densities. For substrate, 0 = control, and 1 = sedimentation treatment.



4.5 DISCUSSION

Sedimentation has been recognised as an important stressor and can elicit multiple ecological impacts on biota directly via abiotic mechanisms and indirectly mediated through the aquatic food web. Whilst studies investigating the ecological effects of sedimentation are numerous, we still know surprisingly little about modified biotic interactions. This chapter quantified effects of sedimentation on predation pressure between a common fish predator and amphipod prey, and demonstrates that sedimentation under low flow conditions increased predator efficiency, resulting in increased proportional consumption of the prey population. These findings highlight the importance of modified biotic interactions in determining prey population size during low flow with and without the added stress of habitat simplification, and suggest that biotic interactions may be an important mechanism underpinning macroinvertebrate assemblage change during natural drought.

In this experiment, the effect of sediment deposition in clogging interstitial spaces and forming an impermeable layer above the original river bed substratum was mimicked using sand as a substitute for gravel and cobbles. Whilst sand was preferential over naturally sourced sediment for the purpose of this feeding experiment, it should be noted that the latter may have influenced the results, e.g. by additionally increasing FPOM which may have altered the behaviour of the amphipod prey, or by adding unknown numbers of eggs and small aquatic larvae such as *Chironomidae* spp., which may have underestimated predatory impacts of bullhead on *G. pulex*. Furthermore, sediment may, in natural systems, enhance macrophyte growth, which has been shown elsewhere to increase habitat complexity and reduce predation (Manatunge *et al*., 2000). Supporting predictions

made in Hypothesis 1, sediment increased the efficiency of the predator at consuming prey, as evidenced by an increased attack rate. Sedimentation also increased handling time coefficient by 7% suggesting that an increased encounter and attack rate increased the proportion of time *C. gobio* were spending processing their prey. This seemingly trivial percentage change was to be expected, as handling time is affected most greatly by predator size and age, the variation of which were minimalised for this experiment. Sedimentation increased proportional consumption compared to the control, indicating that habitat simplification increased the encounters between *C. gobio* and *G. pulex*, the number of attacks by *C. gobio* and the number of attacks that were successful (Fig. C3, Appendix C). This finding, which supports hypothesis two, also highlights the importance of interstitial space as prey refugia in reducing proportional prey consumption. Interstitial refugia has been shown to limit predation of trout eggs by the mottled sculpin (Biga *et al*., 1998) and of salmon eggs by *C. gobio* (Palm *et al*., 2009) due to restricting access to eggs from the predator. However, slimy sculpins have been shown to compress their skulls in order to access interstitial spaces ~20% smaller than their head width (Marsden & Tobi, 2014), thus enabling them to partially overcome barriers to prey encounters in complex habitats. Habitat complexity attributable to interstitial refugia has also proved crucial in determining the functional response in a study by Barrios-O'Neill *et al*. (2015). Increased proportional prey consumption, as evidenced in sediment treatments, could reduce the timescale for prey population destabilisation to occur during natural sedimentation events in streams, increasing the likelihood of local prey extinctions (Reich & Lake, 2015).

Density played an important role in determining the predator impact, and proportional consumption decreased in both substrate treatments as density increased. This illustrates the effect of satiation limiting the number of prey consumption, and thus the more prey available beyond the number of prey that can physically be consumed by one *C. gobio* individual within 24 hours, the smaller the proportional consumption becomes. Functional response curves indicated that this value was approximately 75 individuals of *G. pulex*. This matches the plotted proportional consumption data, which demonstrate a sharp decline in proportional consumption with increasing prey availability at densities > 75. Mottled sculpins have been show to become satiated at ~150 *Baetis* sp. (Soluk, 1993), illustrating the top down predatory impact sculpins can exhibit when confined with an abundant prey item.

Substrate type and initial prey density interacted resulting in differences in proportional prey consumption between substrate treatments at low prey densities, whereas proportional prey consumption at greater prey densities were similar between substrate treatments. This finding demonstrates that prey density was so great as to reach saturation and override the effect of habitat complexity. In other words, habitat complexity effects were overwhelmed by prey densities, resulting in *C. gobio* able to consume prey equally across both substrate treatments. These findings confirm the presence of a type II FR curve in both treatments, as expected, supporting the use of Eq. 1 to quantify attack rate and handling time parameters.

Cottus gobio can typically reduce densities of common stream biota including *Gammarus pulex*, *Baetis rhodani* and *Leuctra* spp. (Dahl, 1998). It is thought that under normal stream flow, prey densities are controlled primarily by prey

movements (i.e. movements between patches, immigration and emigration and drift), and not by consumption by predators (Englund, 2005), though strong top-down controls are found in mesohabitat patches where fish predators forage (Worischka *et al*., 2014). However, findings from this chapter suggest that during sedimentation events coupled with reduced flow and fragmentation of aquatic streambed, prey densities may be governed to a greater extent by predatory impacts, relative to prey movement. This is due to sedimentation (Vadher *et al*., 2015) and fragmentation of the aquatic habitat (Covich *et al*., 2003) restricting taxa movement (Lake, 2003), and due to intensification of predator impacts. Reduced taxa abundance can lengthen the time taken for streams and rivers to recover following disturbance events (Power *et al*., 2008). Findings from this chapter suggest that intensified fish predatory impacts during sedimentation may therefore lengthen the time to ecological restoration following sedimentation, due to lowered macroinvertebrate population size. Predator-prey interactions may even lead to local prey extinctions (e.g. Murdoch & Scott, 1984) further reducing rapid ecological restoration. The experiment has focused on benthic fish predation as pelagic fish are known to be more susceptible to drought and cease feeding at lower elevated temperatures compared to *C. gobio* (Elliott & Elliott, 1995). However, if pelagic fish were able to persist and feed in isolated pools during drought, top-down control exhibited by such taxa could be greater than benthic fish such as *C. gobio*: whilst predatory impacts would be similar when prey were within interstitial refugia inaccessible to their fish predators, prey could be more susceptible to pelagic fish predation that benthic fish predation when moving between interstices, owing to the greater unimpeded field of view of pelagic fish, searching from above the substrate

particles rather than between them, ultimately resulting in a greater prey detection (Dell *et al*., 2014).

Cottus gobio is a searching predator, and the increased predator efficiency gained within the sediment treatment likely reflects a loss of physical and visual barriers, which could otherwise impede searching efficiency by obscuring the sight of predators whilst searching, in turn reducing encounters (Manatunge *et al*., 2000) and attack success (Savino & Stein, 1982). Such habitat complexity effects can govern the FR type (e.g. Hossie & Murray, 2010) in 'sit-and-wait' predators, but are unlikely for foraging fish such as *C. gobio*, particularly when offered a single prey taxa (Murdoch & Bence, 1987). Thus as expected, increased habitat complexity in this study (cobble substrate control) was unable to entirely cease density-dependent predation by *C. gobio* at low densities, but rather reduced the proportional prey consumption (~50%). In agreement, other sculpin species (*Cottus asper*) have been shown to elicit a type II functional response when feeding upon a single prey species (Woodsworth, 1982). Similar findings (using alternative predator and prey taxa) were also found by Alexander *et al*. (2015) mirroring these results. Sculpin predatory impact can also be influenced (e.g. facilitation and interference) by the presence of macroinvertebrate predators (Soluk & Collins, 1988; Soluk, 1993) as well as other sculpins (Fitzsimons *et al*., 2006). Further work could investigate multiple prey and multiple predators, to further mimic the natural conditions found in isolated pools following drought. It is likely that *C. gobio* would switch between prey opportunistically depending on what prey species was most favourable and abundant (Chalupnicki & Johnson, 2016), supporting the notion that the functional response type could change to a type III in the presence of multiple prey species.

Whilst indirect *sedimentation* effects on the predator functional response have not been investigated to date, other forms of habitat complexity have been investigated: for example Diehl (1988) demonstrates macrophytes increase habitat complexity and reduce attack rate and prey consumption by pelagic fish. Similar results have been found also for benthic fish (Kaldonski *et al*., 2008). These studies support findings from this chapter that habitat complexity influences predator interaction strength in fish. Whilst it is possible that sedimentation could mask habitat heterogeneity biotic effects through direct abiotic impacts (Brown, 2007; Peckarsky, 1985), this chapter would suggest sedimentation, through alterations to benthic habitat complexity, can elicit important ecological responses mediated wholly through the aquatic food web (i.e. indirect effects). This experiment revealed changes to aquatic biotic interactions during drought, but aquatic-terrestrial linkages can also be strengthened during drought (Dekar *et al*., 2014) leading to altered biotic interactions both within and across ecosystems (Larsen *et al*., 2015). Such interactions should be carefully considered, as intensified predation of fish by terrestrial predators during drought will clearly have knock on effects on biotic interactions between aquatic organisms within isolated pools.

The experiment outlined in this chapter investigated predator impacts in mesocosms supplied with freshly abstracted groundwater. However, during sedimentation events, specifically those coupled with reduced flow, water quality can rapidly deteriorate adding additional stress to both predators and prey alike. Smothering of prey taxa by sediment may indirectly affect predators through bottom-up control, as predator resources are eliminated by abiotic pressures (Gosselin *et al*., 2010). Ultimately, the fate of remnant macroinvertebrate communities during

sedimentation may depend upon the pervasiveness of abiotic stress, as described by the harsh benign hypothesis (e.g. Menge, 1976). If stress is sufficiently great, top predators which are particularly susceptible to stressors (Petchey *et al*., 1999; Ledger *et al*., 2012) may be extirpated, releasing taxa at lower trophic levels from predation. Conversely, if abiotic conditions following sedimentation are moderately benign, predators are likely to, as illustrated within this study, increase top down control strength on their prey, resulting in indirect biotic effects dominating the fate of the remnant prey community.

4.6 CONCLUSION

Here the importance of biotic interactions in determining prey population size during sedimentation is illustrated by means of a feeding experiment. By utilising a novel approach to increase understanding of the indirect effects invoked by sedimentation, findings illustrate the importance of experiments in determining the mechanistic basis of empirical survey observations. It also opens up many new research questions and further studies should investigate whether *C. gobio* elicit prey switching when offered more than one prey species simultaneously, which may influence the FR curve (Hughes & Croy, 1993; Warburton *et al*., 1998; Leeuwen *et al*., 2007), whether modified taxa velocity attributable to warming (Dell *et al*., 2014) may modify FR parameters (Song & Heong, 1997), and whether habitat size may be important in determining the FR type (Long & Hines, 2012).

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CHAPTER FIVE

Ecological implications of macroinvertebrate physiological responses to warming

5.1 ABSTRACT

Future climate extremes may greatly exacerbate water temperatures, which in turn may exceed activity thresholds of aquatic biota. The occurrence of elevated but non-lethal temperatures may have wide ranging ecological effects on functional processes such as predation, grazing and decomposition, but research on the activity thresholds of macroinvertebrates is scarce. Moreover, few river water-temperature datasets incorporating extreme events exist, and thus it remains to be seen whether warming of lowland rivers may exceed physiological limits of macroinvertebrates in nature. In this chapter, the warming tolerance of 28 chalk stream macroinvertebrate taxa was investigated, by comparing their activity thresholds (including CTmax and Heat Coma) with river water temperatures for a range of lowland streams with contrasting hydrological regimes. Mean CTmax varied greatly among taxa, ranging from 22.0 °C (*Rhyacophila dorsalis*) to 37.3 °C (*Ceratopogonidae*), as did heat coma, whilst activity threshold plasticity increased with increasing sensitivity (i.e. lower CTmax). Respiratory mode helped explain thermal activity threshold differences among taxa. During summer months, water temperatures of flowing streams reached 21.1 °C – approaching yet not exceeding the CTmax of any taxa investigated, whereas stagnant stream pool temperatures reached 31.1 °C – exceeding the CTmax of 50% of taxa investigated. Physiological diversity within groups should allow functioning to persist, although differential activity thresholds between prey and their predators may have indirect effects upon community structure and functioning. The findings illustrate how compound thermal disturbances have the potential to exceed physiological tipping points of biota and functional processing, and highlights the importance of physiological thresholds as a mechanism underpinning ecological responses to extreme warming.

5.2 INTRODUCTION

Climate change has increased global surface temperatures by 0.85°C over the last 130 years (IPCC, 2013), prompting a wave of new studies to understand the ecological impacts of global warming (e.g. Worthington *et al*., 2015) and biotic responses to mean temperature change (e.g. Hogg *et al*., 1995; O'Gorman *et al*., 2014). Increases in mean water temperature (Hannah & Garner, 2015) are expected to continue in line with surface air temperatures (Chessman, 2009; Houghton & Shoup, 2014). Coupled climate-hydrology models also predict that extreme events such as heat waves and hot days will increase in frequency in the future (Beniston *et al*., 2007; Verdonschot *et al*., 2015), and may co-occur with drought (Arismendi *et al*., 2013) as compound events that strongly exacerbate the variability of river water temperature (Van Vliet *et al*., 2011). Hydrologic drought leads to flow cessation and the fragmentation of river channels into isolated pools (Boulton, 2003; Larned *et al*., 2010), and can also cause marked temperature fluctuations in the remaining pool water (Mundahl, 1990). Whilst most species are well adapted to temperature regimes that fall within the bounds of normal variability, amplified temperature variability experienced by biota during rare extreme events may have profound consequences for biodiversity and ecosystem functioning.

Temperature is one of the most important abiotic variables responsible for regulating physicochemical processes and can govern the metabolic rate (Gillooly *et al*., 2001; Brown *et al*., 2004), growth (Pockl, 1992; Suhling *et al*., 2015), mortality (Tramer, 1977), feeding (Maltby *et al*., 2002) and fecundity (Pritchard *et al*., 1996) of aquatic ectotherms, as well as community composition (Burgmer *et al*., 2007). Effects at the community level are most likely driven by impacts at the individual

level, governed by biological traits such as thermal sensitivity (Dallas & Rivers-Moore, 2014). Temperature can also alter the solubility and respiratory demands of oxygen required by biota (Verberk *et al*., 2011), and may interact with other stressors to exacerbate their effects (Brook *et al*., 2008; Laetz *et al*., 2014). Climate warming may thus have critical implications for aquatic macroinvertebrates, especially during extreme events when temperature fluctuation is exacerbated. Many studies investigating organism's sensitivity to temperature have been driven by thermal discharges in rivers from power stations (Worthington *et al*., 2015), but such knowledge pertaining to thermal sensitivity may also help to predict physiological and ecological responses to future global warming (Dallas & Ross-Gillespie, 2015). Despite the pervasive role temperature will likely have upon aquatic animals in future, thermal activity thresholds have mostly focused on fish (e.g. Anttila *et al*., 2013; Beitinger & Lutterschmidt, 2011; Dent & Lutterschmidt, 2003; Fischer & Schlupp, 2009; Rajaguru & Ramachandran, 2001) and the activity thresholds of aquatic macroinvertebrates remain largely unknown.

Some macroinvertebrates can escape drought by emigrating in response to environmental cues such as declining water depth and/or rising water temperature (Velasco & Millan, 1998). Where elevated water temperatures and desiccation are seasonally predictable, aquatic organisms have developed mechanisms such as aerial life stages (Hynes, 1970; Larned *et al*., 2010; Masters *et al*., 2007) to avoid stress, and often the community assemblage may be significantly different to systems with a lower thermal predictability (Eady *et al*., 2013). However the only viable option for many species is to simply tolerate conditions in river channels as they dry and fragment. Remnant pools have the potential to act as refuges during

drought (Scheffers *et al*., 2014), depending on the physical and chemical properties of the remaining habitat, and the physiological tolerance of the remnant organisms (Magoulick & Kobza, 2003). In many instances however, drought leads to high mortality in many macroinvertebrate groups (Verdonschot *et al*., 2015), although the underlying causative mechanism and source of the stress (e.g. high temperature, low oxygen, smothering by sediment) remains uncertain.

Elevated temperatures can result in three main organismal responses: a loss of movement, a loss of metabolic functioning, and mortality (Bailey, 1955; Cottin, *et al*., 2012; Dallas & Ketley, 2011; Dallas, 2008; Das *et al*., 2005; Miller & Stillman, 2012; Nelson & Hooper, 1982; Terblanche *et al*., 2005; Vorhees & Bradley, 2012). To measure lethal temperatures (i.e. thermal tolerance), survival can be assessed by exposure to a range of elevated dynamic or static temperatures over a set period of time (e.g. Cox & Rutherford, 2000; Dallas & Ketley, 2011; Fischer & Vasconcellos-Neto, 2003; Pandolfo *et al*., 2010; Sinclair *et al*., 2006). However to evaluate activity thresholds prior to extirpation, taxa must be subjected to ramping temperatures and their movement recorded, to determine phenotypic responses to elevated, yet sub-lethal temperatures (e.g. Cottin *et al*., 2012; Everatt *et al*., 2013; Hazell *et al*., 2008; Hughes *et al*., 2010; Owen *et al*., 2013). Such methods, e.g. the critical thermal method (CTM), have been given much praise to determine impacts of environmental change (Terblanche *et al*., 2011). Commonly used phenotypes adopted by physiologists in CTM experiments are the critical thermal maximum (CTmax) and heat coma (HC). CTmax is defined as "*the thermal point at which locomotory activity becomes disorganised and the animal loses its ability to escape from conditions that will promptly lead to its death*" (Cowles & Bogert, 1944), and

HC is described as the temperature where all movement ceases and mortality quickly ensues (Chown & Nicolson, 2004). These phenotypic traits are important in determining biotic response to extreme climatic events (Chessman, 2015; Somero, 2010). As HC occurs near to an organism's physiological death, this phenotype can provide an indication of thermal tolerance. On the other hand, CTmax, which can occur at temperatures much lower than HC and is typically reversible, can provide an indication of when biotic functioning may temporarily cease. Variability in these activity thresholds may relate to traits such as rheophily and respiration (Chessman, 2015), whilst intra-species tolerance plasticity to elevated temperatures may depend on overall thermal tolerance, as outlined by the 'trade-off hypothesis', which states that tolerance plasticity is reduced as overall tolerance is increased (Gunderson & Stillman, 2015). It is likely that these thermal activity thresholds may determine a macroinvertebrate's ability to undertake specific ecosystem processes, and thus influence the strength of biotic interactions in the wider food web.

Macroinvertebrates perform key functional processes within streams and rivers (Graca, 2001; Petersen & Cummins, 1974) and are integral in the provisioning of ecosystem services (Millenium Ecosystem Assessment, 2005). Macroinvertebrates may be grouped into functional feeding groups (FFG; Cummins, 1973; Cummins & Klug, 1979), which define their principal mode of feeding and ability to perform functional roles. However, warming may alter an organism's activity which in turn may govern the functional processing rates mediated by biota (Dang *et al*., 2009). Where temperatures exceed activity thresholds of predatory taxa but not their prey, consumption of prey organisms may cease (Elliott & Elliott, 1995) releasing prey from top-down control, and may invoke 'physiological depression' of predator

impacts (reduced feeding owing to thermal stress; Kishi *et al*., 2005). Moreover, warming may alter processing of basal resources, which may have repercussions for functional processes such as nutrient cycling (Maltby *et al*., 2002). Thus temperature may invoke indirect, secondary effects on stream ecology that may help explain empirical, ecological (community structure and functioning) responses to warming. However to date, most insights are only provided by the terrestrial literature, predominantly focusing on the activity thresholds of biocontrol agents and their pest prey (e.g. Coombs & Bale, 2013; Hughes *et al*., 2010a; Hughes *et al*, 2010b), and a lack of detailed understanding of how warming will affect ecological processes in streams currently hampers efforts to predict how extreme events will affect biotic and abiotic parameters in running waters (Hutchins *et al*., 2016).

Studies that have investigated lethal temperatures to macroinvertebrates have determined the *survival* of macroinvertebrates to warming (e.g. Quinn *et al*., 1994; Stewart *et al*., 2013). Others have taken a macroecological approach by determining the distribution of taxa based on their thermal sensitivity and future climate ('climate matching') (e.g. Hering *et al*., 2009), but such studies often focus on terrestrial organisms along with mean, annual, surface air temperatures (e.g. Deutsch *et al*., 2008). However direct extirpation from high temperature may only partly explain modified macroinvertebrate assemblages and functional processing rates. Temperate regions such as the U.K. for example may rarely experience lethal water temperatures but may frequently experience elevated, yet sub-lethal temperatures, particularly during extreme events such as drought, hot days and heat waves. Therefore the ability of taxa to move and perform functions at elevated temperatures may be of greater ecological relevance than direct mortality *per se*,

and hence methods capable of determining activity thresholds in response to elevated, sub-lethal temperatures may be crucial in predicting future ecological effects of warming. Historically it was believed CTmax occurred only at temperatures beyond what organisms could be exposed to in nature, and thus the importance of CTmax was only to make comparisons between taxa (Houghton *et al*., 2014). However, with extreme events projected to increase in severity and occur concurrently as compound events (Arismendi *et al*., 2013), river water temperatures are expected to increase (Guan *et al*., 2015; Hannah & Garner, 2015) and therefore the physiological impact of elevated water temperatures must be explored.

In order to explain and predict ecological responses to warming, attributable to extreme events, we must also understand the typical range of warming that lowland river waters may experience, yet such knowledge remains sparse. Water temperature can vary predictably according to diel and seasonal cycles, but the extent of this variation can interact critically with local climate, weather, shading and river flow (Broadmeadow *et al*., 2011; Rutherford *et al*., 2004; Van Vliet *et al*., 2011; Webb, 1996). Environmental agencies fail to pick up extreme water temperatures in remnant water pockets during hydrologic drought as fixed monitoring stations become exposed to air once water depth falls. Additionally, most research investigating stream and river temperature often either use mean temperature values of running waters (e.g. Huguet *et al*., 2008; Webb & Nobilis, 2007) or model running water temperatures using mean air temperature values (e.g. Durance & Ormerod, 2007). Many studies pertaining to river water temperature are often primarily interested in changes to flowing systems under different climatological scenarios (e.g. Huguet *et al*., 2008b; Omid *et al*., 1999; Webb, 1996) or moderate

discharge reductions (Van Vliet *et al*., 2011). However, studies reporting extreme water temperatures, for example in stagnant, fragmented pools, are often opportunistic (e.g. Tramer, 1977) and thus generally scant.

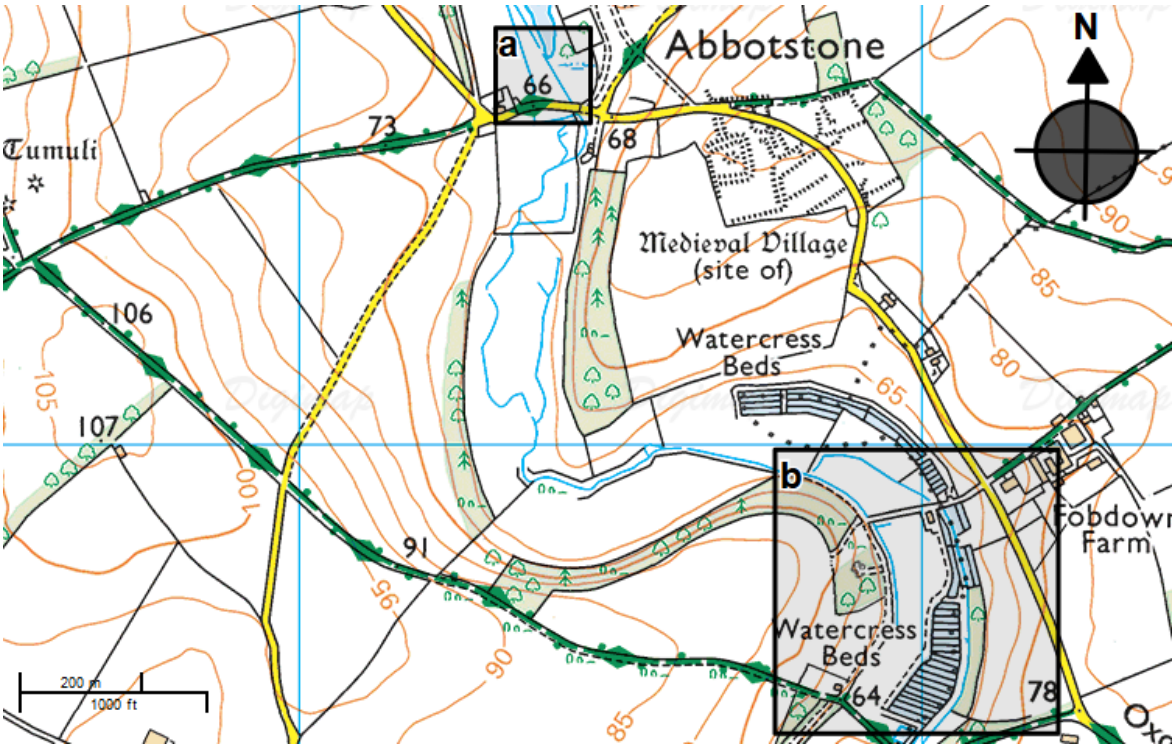
In this chapter, chalk stream macroinvertebrate activity thresholds are determined and compared to lowland river water temperatures under a range of hydrological conditions. This in turn provides an idea of macroinvertebrate warming tolerance (described as the difference between CTmax and maximum environmental temperature, (Duarte *et al*., 2012; Richter-Boix *et al*., 2015) to future events. Chalk streams were investigated as these systems are predominantly groundwater fed (Wood & Petts, 1999) and therefore – at present – thermally stable (Berrie, 1992), thus making them particularly sensitive to future global change if groundwater inputs are reduced. These river systems also occur primarily in southern England where extreme events such as drought and hot days are predicted to increase (Vidal & Wade, 2009). The aim of this study was to determine how stream water temperature may affect thermal tolerance (HC [direct effect; near-physiological death]) and biotic functional processes (CTmax [indirect effect; cessation of normal activity]) during extreme hydrological events. This is achieved by 1) developing a database of activity threshold phenotypes for chalk stream macroinvertebrates, 2) assessing lowland river temperatures under different hydrological conditions and 3) comparing derived activity thresholds to stream water temperatures.

5.3 METHODOLOGY

5.3.1 Macroinvertebrate collection and housing

Macroinvertebrates were obtained by kick sampling (1 mm mesh) in water courses of a watercress farm and an adjacent chalk stream in New Alresford, Hampshire U.K. (51°6’4”N, 1°11’13”W; Fig. 5.1). Where possible, macroinvertebrates were obtained from a single location (feeder channel) to reduce physiological plasticity caused by differential thermal history (Farrell *et al*., 2008). As organism size has been shown to influence activity thresholds (Buchanan *et al*., 1988), individuals of a visually similar size for each taxon were selected for use in warming experiments. Macroinvertebrate collection took place weekly during summer and autumn months, in order to limit the time taxa were held in cold room storage prior to experimentation.

Figure 5.1. **Map of macroinvertebrate collection sites at Fobdown Farm, Alresford, U.K**. Square 'a' shows the sampling reach of the Candover Brook, a small headwater chalk stream. Square 'b' shows the location of a drainage channel. Source: Ordnance Survey (Digimap Licence) 2016.



Macroinvertebrates were picked from kick samples, transferred to 500 ml wide-mouth bottles containing local river water, transported to the University of Birmingham in cool boxes, stored in a 10 °C constant temperature room with a L:D 12:12 regime after Dallas & Rivers-Moore (2012), and starved (by removing resources) for 24 hours to limit digestive effects on activity thresholds (Dallas & Ketley, 2011). The cold room temperature (~10 °C) was very similar to that of upwelling groundwater feeding the source streams (10.319 ± 0.0002 °C; autumn 2012 to summer 2013 data) and within the annual temperature range of the Candover Brook, a nearby groundwater-dominated headwater chalk stream (mean winter temperature 7.4 °C ± 0.02 and mean summer temperature 16.8 °C ± 0.03).

5.3.2 Critical Thermal Method (CTM)

A CTM approach (Hazell *et al*., 2008) was used to determine the thermal limits of activity of 28 macroinvertebrate species characteristic of lowland chalk streams, specifically mayflies (n = 5), stoneflies (n = 2), caddisflies (n = 4), beetles (n = 2), damselflies (n = 1), alderflies (n = 1), true flies (n = 6), crustaceans (n = 2), molluscs (n = 2), flatworms (n = 1) and Annelida (n = 2); Table 5.1.

Table 5.1. **List of macroinvertebrate taxa investigated in the warming experiment**. n = total number of individuals tested, n/run = number of individuals per run. FFG = Functional Feeding Group, Resp = mode of respiration, Size = maximum potential size, Disp = dispersal mechanism and Cycle = number of annual generational cycles. Taxa arranged alphabetically by Order.

Taxon	n	n/run	FFG	Resp	Size (cm)	Disp	Cycle
Gammarus pulex	18	3	Shredder	Gill	1-2	Aquatic	>1
Elmis aenea (adult)	11	5-6	Grazer	Plastron	0.25-0.5	Aquatic Terrestrial	1
Elmis aenea (larvae)	7	7	Grazer	Gill	0.25-0.5	Aquatic Terrestrial	1
Limnius volckmari (adult)	8	8	Grazer	Plastron	0.25-0.5	Aquatic Terrestrial	1
Limnius volckmari (larvae)	14	7	Grazer	Gill	0.25-0.5	Aquatic Terrestrial	1
Anopheles plumbeus	14	6-8	Grazer	Spiracle	0.5-1	Aquatic Terrestrial	>1
Ceratopogonidae	13	6-7	Predator	Gill	1-2	Aquatic Terrestrial	>1
Dicranota	14	4-5	Predator	Spiracle	2-4	Aquatic Terrestrial	1
Macropelopia	16	4-6	Predator	Tegument	0.5-1	Aquatic Terrestrial	1
Micropsectra	17	5-6	Collector	Tegument	0.5-1	Aquatic Terrestrial	>1
Tipula (*Arctotipula*)	10	2-3	Shredder	Spiracle	2-4	Aquatic Terrestrial	1
Baetis rhodani	18	3	Grazer	Gill	0.5-1	Aquatic Terrestrial	>1
Caenis luctuosa	8	3-5	Collector	Gill	0.5-1	Aquatic Terrestrial	>1

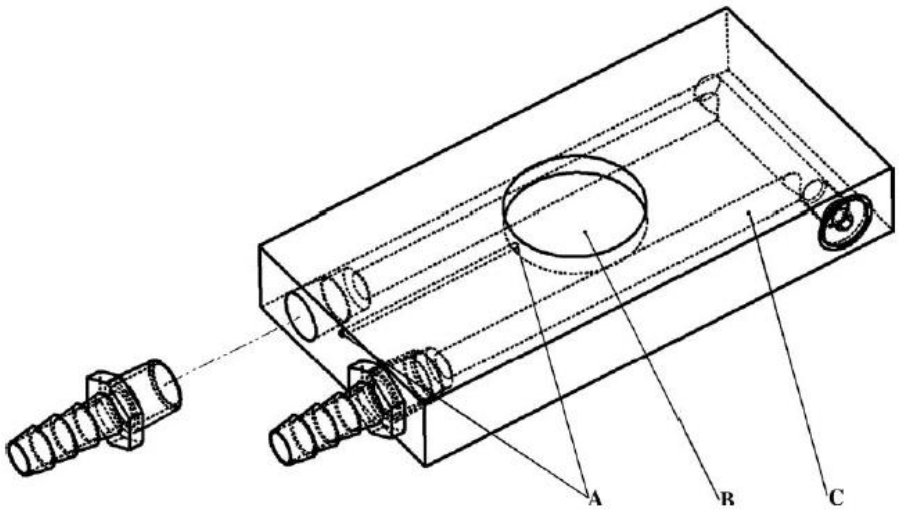
Table 5.1 continued. **List of macroinvertebrate taxa investigated in the warming experiment**. n = total number of individuals tested, n/run = number of individuals per run. FFG = Functional Feeding Group, Resp = mode of respiration, Size = maximum potential size, Disp = dispersal mechanism and Cycle = number of annual generational cycles. Taxa arranged alphabetically by Order.

Ephemera danica	18	3	Collector	Gill	2-4	Aquatic Terrestrial	<1
Heptagenia sulphurea	16	3-4	Grazer	Gill	1-2	Aquatic Terrestrial	1
Serratella ignita	18	3	Collector	Gill	0.5-1	Aquatic Terrestrial	1
Tubificidae	12	6	Collector	Tegument	4-8	Aquatic	>1
Ancylus fluviatilis	17	5-6	Grazer	Tegument	0.5-1	Aquatic	1
Radix balthica	18	5-6	Grazer	Tegument	1-2	Aquatic	1
Asellus aquaticus	18	3	Collector	Gill	1-2	Aquatic	>1
Sialis lutaria	18	2-3	Predator	Gill	1-2	Aquatic Terrestrial	<1
Caolpteryx virgo	18	1-2	Predator	Gill	2-4	Aquatic Terrestrial	1
Leuctra fusca	7	3-4	Collector	Tegument	0.5-1	Aquatic Terrestrial	1
Nemurella picteti	18	3	Collector	Tegument	0.5-1	Aquatic Terrestrial	1
Helobdella stagnalis	15	4-6	Predator	Tegument	0.5-1	Aquatic	1
Polycelis nigra	18	3	Predator	Tegument	1-2	Aquatic	1
Agapetus fuscipes	18	9	Grazer	Tegument	0.5-1	Aquatic Terrestrial	1
Drusus anulatus	18	3	Grazer	Tegument	1-2	Aquatic Terrestrial	1
Hydropsyche pellucidula	18	3	Filterer	Gill	1-2	Aquatic Terrestrial	1
Rhyacophila dorsalis	18	3	Predator	Gill	1-2	Aquatic Terrestrial	1

The CTM apparatus consisted of an aluminium block containing a milled arena (diameter 40 mm; depth 15 mm; volume 20 ml) for stream water and test organisms (Fig. 5.2b), a drilled channel that received re-circulated antifreeze (Fig. 5.2a) from a temperature controlled water bath (Haake Phoenix 11 P2, Thermo Electro Corp.,

Germany), and a drilled channel (Fig. 5.2c) allowing a K-type submersible thermocouple probe to enter the arena through the side wall to measure water temperature. The probe connected to a Tecpel 315 type K thermometer unit (Tecpel, Taiwan) which in turn connected to a central PC. A video camera (Infinity 1-1; Lumenera Scientific, Canada) with a macro lens (Computar MLH-10X, CBC Corp., New York, NY) along with Studio-Capture DT and Studio-Player software (Studio86Designs, U.K.) was used for video capture and playback.

Figure 5.2. **Diagram of apparatus used in CTM trials**. Diagram illustrates A) thermocouple probe entry, B) milled arena, and C) alcohol transport channels sat beneath the arena. Diagram sourced from Hazell *et al*. (2008).



Individuals were placed within an arena containing river water / treated tap water, and following five minutes acclimation from handling stress (Terblanche & Chown, 2007; Bury, 2008; Lyons *et al*., 2012 and Hazell *et al*., 2010) the water temperature was increased at a constant rate (0.2 °C min^{-1}) consistent with published protocols

(Everatt *et al*., 2014; Owen *et al*., 2013) up to 45 °C, or until HC had been reached. This rate of warming is sufficient to prevent acclimation, yet enables the body temperature of study animals to equilibrate with the water medium (Dallas & Rivers-Moore, 2012). Whilst lower warming rates are more likely to be experienced in the field, faster ramping rates may be experienced under 'extreme conditions' and are thus ecologically justifiable (Terblanche *et al*., 2011). Dissolved oxygen within the arena was maintained above 70% saturation by aeration (Dallas & Ketley, 2011). The number of individuals per run was dependent on the size of the taxon investigated (Table 5.1).

Video playback identified a multitude of phenotypes (Table D1, Appendix D) including CTmax and HC. Exact CTmax phenotype activity varied among taxa, but were all consistent in that they represented the temperature at which locomotory coordination became uncontrolled. HC was determined by the final movement of an organism's appendage, or the final movement of the body for those without. For one species representing each of collectors (*Asellus aquaticus*), shredders (*Gammarus pulex*), grazers (*Radix balthica*) and predators (*Calopteryx virgo*) (total species n = 4), phenotypes in addition to HC and CTmax were recorded (loss of grip, final movement in water column, abdominal twitching) which were thought to represent the onset of stress and cessation of functioning at elevated temperatures prior to the expression of CTmax.

5.3.3 Water temperature variation in lowland streams

To determine whether lowland stream water temperature could exceed activity thresholds (i.e. CTmax and HC) of chalk stream macroinvertebrates, temporal variation within time series data from a range of natural locations and experiments were described and compared with a range of activity threshold data obtained for lowland macroinvertebrates. Six time series were analysed, capturing water temperature variation during normal flow (River Lambourn, Candover Brook, U.K.), reduced flow (Winterbourne Stream, U.K.), a heat wave (River Itchen, U.K.) and during a naturally occurring drying pool (River Teme, U.K.) and simulated drought (NERC DriStream mesocosm experiment, U.K.). Details of each of these data sources are summarised in Table 5.2.

At all sites, Tinytag Aquatic TG-4100 (Gemini Data Loggers Ltd., Chichester, U.K.) loggers were used. Time series data were converted to frequency distribution in R, version 3.2.0 (R Core Development Team) using the package ‘ggplot2’, and plotted.

Table 5.2. **Stream location and temperature summary data.** Showing information for data sources used to compare against derived macroinvertebrate activity thresholds.

Watercourse	Location	Logger resolution	Duration	Notes
River Lambourn	51°26'30"N, 1°22'34"W	1 hour^{-1}	May to October, 2012	
Winterbourne Brook	Honeybottom (51°25'50"N, 1°20'43"W) Bagnor (51°25'30"N, 1°21'5"W)	1 hour^{-1}	May, 2012	
River Itchen	50°57'4"N, 1°20'32"W	4 hour^{-1}	June to October, 2015	Capturing record hot day (1st July; 36.7 °C at Heathrow (~50 miles away)
River Teme	52°21'26"N, 2°52'46"W	30 hour^{-1}	July, 2013	Warm month (bank-side day-time [9am – 9pm] mean air temperature = 23.1 °C ± 0.16, min = 16 °C, max = 36.9 °C)
'DriStream mesocosms'	51°6'4"N, 1°11'13"W	4 hour^{-1}	July to August, 2014	Artificial stream channels, supplied with abstracted groundwater. Control channel (water depth = 35 cm) and a drought treatment channel (water depth = 7 cm).
Candover Brook	51°6'4"N, 1°11'13"W	4 hour^{-1}	December 2012 to February 2013 (winter) and July to August 2013 (summer)	

5.3.4 Comparing taxa activity thresholds to river temperatures

The range of CTmax for taxa derived from the laboratory CTM, as per above, were then compared to lowland stream water temperature, derived from the time series datasets. CTmax provided an activity threshold whereby cessation of functioning could be assumed but survival was still likely following a return to cooler temperatures. CTmax were compared with river water temperatures under normal flow, and under stagnant conditions. Additionally, taxa were grouped by functional feeding group (FFG) to determine the proportion of each group that may temporarily become 'functionally impaired' (i.e. where CTmax < water temperature), and by 'prey' (i.e. primary consumers) or 'predators' (i.e. secondary consumers) to assess the potential consequences of water temperature on biotic interactions and food web vulnerability under different hydrological and thermal regimes. Finally, taxa were grouped by mode of respiration, maximum potential body size, dispersal mechanism and number of generational cycles, using published traits by Usseglio-Polatera (1991) and Chevernet *et al*. (1994) to investigate possible causative mechanisms underpinning observed taxa physiological thresholds.

5.4 RESULTS

5.4.1 Macroinvertebrate activity thresholds

Mean CTmax values amongst taxa ranged from 22.0 °C to 37.3 °C (Table 5.3). The four greatest mean CTmax values were for Diptera, specifically *Ceratopogonidae* sp. (37.3 °C), *Anopheles plumbeus* (36.2 °C) and *Tipula* sp. (36.0 °C), and Coleoptera (*Limnius volckmari* (Adult); 35.7 °C). The four lowest mean CTmax values were for Trichoptera, namely *Rhyacophila dorsalis* (22.0 °C), *Hydropsyche*

pellucidula (25.5 °C) and *Agapetus fuscipes* (25.8 °C), and Ephemeroptera (*Caenis luctuosa*; 25.1 °C).

Intra-variability was also observed within species and varied considerably between different taxa. Coefficient of variation and CTmax were strongly correlated (linear regression; R^2 = 0.69, F = 66.63, *P* <0.001), which demonstrated that a greater CTmax reduced intra-taxon thermal plasticity. Taxa with the lowest coefficient of variance were *Elmis aenea* (adult = 0.3; CTmax = 34.8 °C, larvae = 0.6; CTmax = 32.8 °C), *Ancylus fluviatilis* (0.4; CTmax = 34.9 °C) and *Gammarus pulex* (1.8; CTmax = 32.4 °C). Taxa with the greatest CTmax variability were *Rhyacophila dorsalis* (15.6; CTmax = 22.0 °C) and *Baetis rhodani* (12.6; CTmax = 25.1 °C).

HC also varied among taxa, and those with the highest HC temperatures were for Diptera (*Ceratopogonidae* sp. (40.8 °C)), Mollusca (*Radix balthica* (40.8 °C)) and Megaloptera (*Sialis lutaria* (40.7 °C)). The lowest HC temperatures were for Trichoptera (*Rhyacophila dorsalis* (24.6 °C)) and Ephemeroptera (*Baetis rhodani* (25.7 °C)). The difference between CTmax and HC (i.e. HC minus CTmax) amongst taxa ranged from 0.6 °C (*Baetis rhodani*) to 9.4 °C (*Agapetus fuscipes*), and averaged (mean) 4.71 ± 0.44 °C across all taxa.

Table 5.3. **Mean CTmax and HC values for each of the 28 taxa investigated**. Taxa ordered alphabetically by major taxonomic group (Annelida – Tricladida). CTmax = Critical Thermal Maximum, HC = Heat Coma, and Coefficient of Variation = the degree of variation in measurements within each taxon.

Taxon	CTmax (°C)	SE	Coeff. of Var. (CV %)	HC (°C)	SE
Helobdella stagnalis	31.6	0.6	7.6	40.0	0.2
Tubificidae	29.8	0.5	6.3	38.9	0.4
Asellus aquaticus	30.2	0.3	4.6	36.1	0.2
Gammarus pulex	32.4	0.1	1.8	35.1	0.2
Elmis aenea (adult)	34.8	0.3	0.3	37.7	0.2
Elmis aenea (larvae)	32.8	0.6	0.6	38.6	0.1
Limnius volckmari (adult)	35.7	0.2	1.5	36.5	0.8
Limnius volckmari (larvae)	30.7	0.4	5.3	36.5	0.5
Anopheles plumbeus	36.2	0.2	2.2	37.7	0.1
Ceratopogonidae	37.3	0.3	2.5	40.8	0.4
Macropelopia	32.1	0.4	5.3	36.4	0.2
Micropsectra	31.5	0.5	7.0	37.3	0.2
Dicranota	31.4	0.4	4.9	34.6	0.3
Tipula (*Arctotipula*)	36.0	0.3	2.8	39.4	0.3

Table 5.3 continued. **Mean CTmax and HC values for each of the 28 taxa investigated**. Taxa ordered alphabetically by major taxonomic group (Annelida - Tricladida).

Baetis rhodani	25.1	0.7	12.6	25.7	0.8
Caenis luctuosa	30.6	0.6	5.7	38.0	0.2
Ephemera danica	34.2	0.4	4.4	38.7	0.2
Heptagenia sulphurea	30.2	0.6	7.3	32.3	0.5
Serratella ignita	29.8	0.5	7.8	35.0	0.3
Sialis lutaria	31.4	0.6	7.5	40.7	0.2
Ancylus fluviatilis	34.9	0.4	0.4	37.9	0.2
Radix balthica	34.3	0.4	5.1	40.8	0.2
Caolpteryx virgo	34.2	0.4	4.5	38.9	0.4
Leuctra fusca	30.1	0.5	4.4	34.8	0.2
Nemurella picteti	30.1	0.3	4.5	33.1	0.2
Agapetus fuscipes	25.8	0.4	6.6	35.2	0.3
Drusus anulatus	28.8	0.4	5.8	33.8	0.2
Hydropsyche pellucidula	25.5	0.5	8.8	32.3	0.2
Rhyacophila dorsalis	22.0	0.8	15.6	24.6	1.2
Polycelis nigra	28.2	0.6	8.9	31.7	0.3

Table 5.4. **Pre CTmax phenotype descriptions**. Descriptions and values given for a common taxon spanning four functional feeding groups. Functional feeding groups ordered alphabetically. FFG = Functional Feeding Group and CTmax = Critical Thermal Maximum.

FFG	Taxa	Pre CTmax phenotype description*	Mean value (°C)	SD	Difference (CTmax - pre CTmax phenotype)
Collector	*Asellus aquaticus*	Final attempt to grip vertical surface	27.7	1.64	2.5
Grazer	*Radix balthica*	Final attempt to grip vertical surface	33.8	1.16	0.5
Predator	*Calopteryx virgo*	'Abdominal flicking'	21.1	1.66	13.1
Shredder	*Gammarus pulex*	Final movement within the water column	31.0	0.75	1.4

*see Table D1, Appendix D for more detailed descriptions

5.4.2 Lowland stream water temperature

Water temperature varied considerably among lowland streams depending on hydrological regime (e.g. normal flow, reduced flow, stagnation; as well as distance downstream) and time of year (summer vs. winter). Mean water temperature ranged from 7.40 °C to 21.03 °C, and maximum water temperature ranged from 10.25 °C to 31.11 °C (Table 5.5; Figs. 5.3-5.5). The River Itchen logging period encompassed a heat wave (Met Office, 2015).

Table 5.5. **Lowland stream water temperature summary statistics**. Third column from the left denotes corresponding figure.

Source		Fig.	Mean (°C)	SE	Median (°C)	Minimum (°C)	Maximum (°C)
River Lambourn		5.3a	12.25	0.022	12.30	8.38	17.57
Winterbourne Stream	Upstream	5.3b	11.28	0.068	11.14	7.68	16.33
	Downstream	5.3b	11.98	0.086	11.63	7.98	19.09
Candover Brook	Summer	5.4a	16.88	0.035	16.51	13.48	20.85
	Winter	5.4a	7.40	0.018	7.34	4.80	10.27
River Itchen		5.4b	15.45	0.017	15.57	10.75	21.09
DriStream mesocosms	Control	5.5a	10.69	0.005	10.58	10.27	11.76
	Drought	5.5a	12.14	0.027	11.73	9.18	17.45
River Teme		5.5b	21.03	0.055	19.92	11.00	31.11

Figure 5.3. **Density plots illustrating temperature variability distribution** for a) River Lambourn (May-Oct, 2012) and b) Winterbourne Brook (May, 2012). Vertical dashed lines illustrate the range of CTmax across all 28 taxa investigated. N.B different y axis scales.

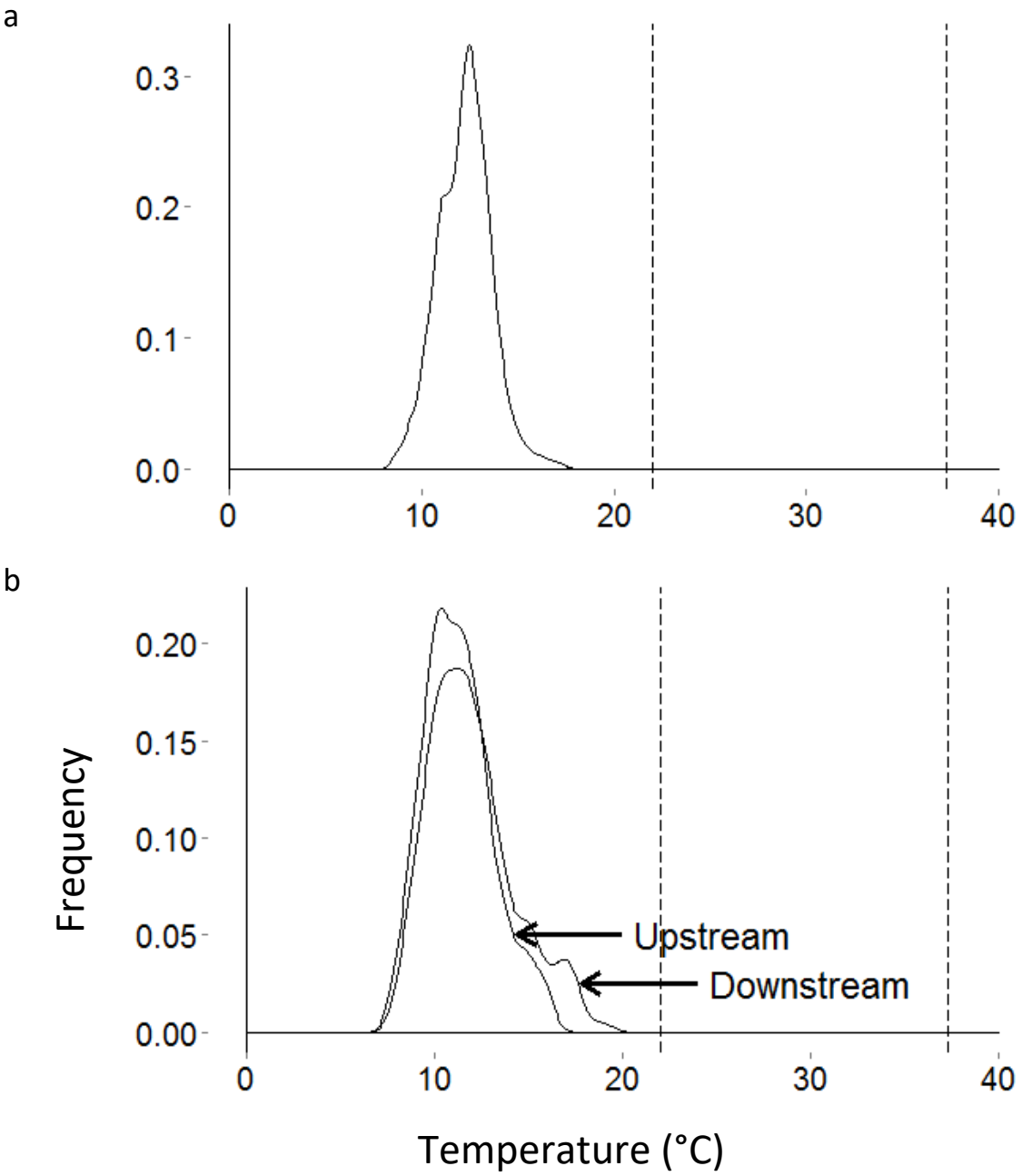


Figure 5.4. **Density plots illustrating temperature variability distribution** for a) Candover Brook (winter = Dec-Feb, winter = July-Aug, 2012-2013); b); River Itchen (June-Oct, 2015 [arrow indicates max temperature obtained on 1st July hot day]); Vertical dashed lines illustrate the range of CTmax across all 28 taxa investigated. N.B different y axis scales.

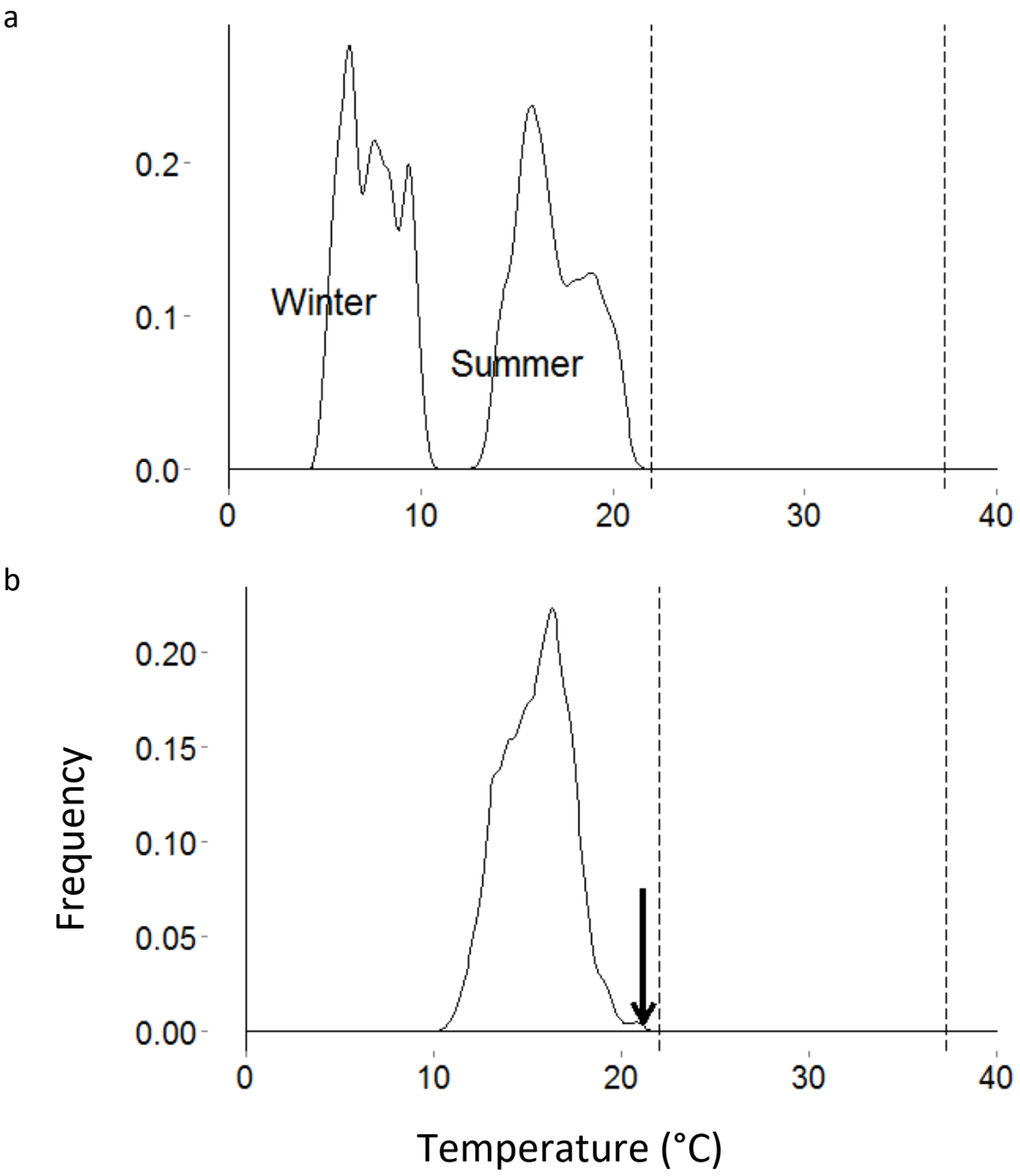
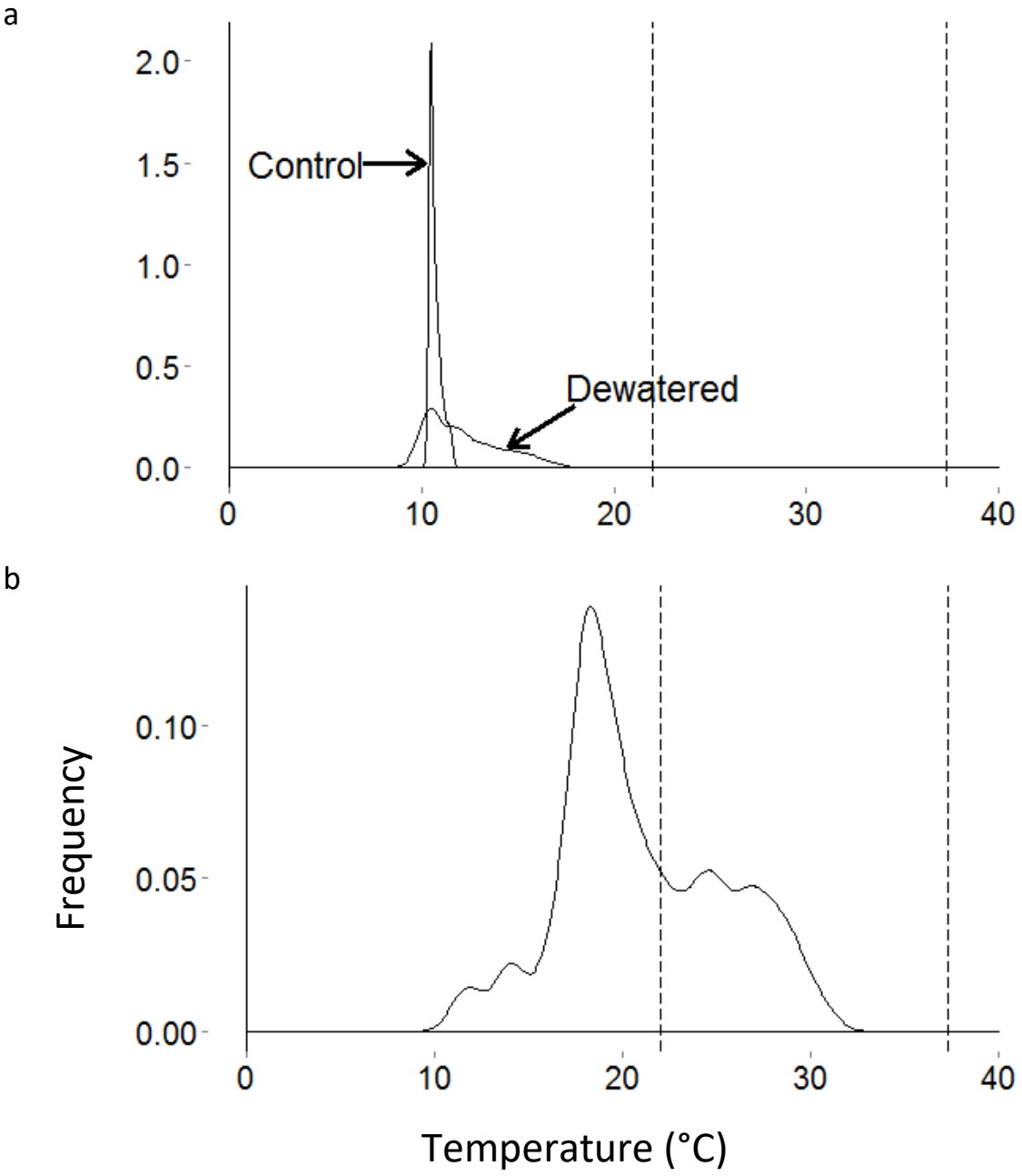


Figure 5.5. **Density plots illustrating temperature variability distribution** for a) Mesocosm channels (July-Aug, 2014) and b); River Teme (July, 2013). Vertical dashed lines illustrate the range of CTmax across all 28 taxa investigated. N.B different y axis scales.



5.4.3 Stream community structure and functioning vulnerability

Section 5.4.1 illustrated that CTmax was highly variable among taxa, whilst section 5.4.2 illustrated that water temperature in lowland streams can also be highly variable based on factors such as hydrological regime and local climate. Therefore, the ability of an organism to undertake a function depends on a) its specific activity threshold(s), and b) the temperature of the water medium which it is within. Rather than looking at taxa independently of one another, taxa can be assembled into functional feeding groups, allowing physiological effects at the individual level to be determined at a higher, and more meaningful, level of ecological complexity.

Water temperatures in flowing rivers often approached but never exceeded the CTmax of the 21 chalk stream taxa investigated. Conversely, water temperature in remnant pools during drought exceeded the CTmax of 50% of taxa. When grouped by FFG, 0% (shredders), 25% (predators), 56% (grazers), 75% (collectors) and 100% (filterers) had a CTmax lower than the maximum stagnant pool water temperature (Figs. 5.6-5.7).

Zero percent of both primary consumers (i.e. collectors, grazers, shredders and filterers) and secondary consumers (i.e. 'predators') had a CTmax less than maximum running water temperature (21.1 °C). Primary consumers would be disproportionately negatively affected by stagnation (+25%) as 50% of primary consumers compared to just 25% of secondary consumers exhibited a CTmax less than maximum stagnant pool water temperature (31.1 °C). A further warming of 4 °C (in line with predictions for the end of the century (IPCC, 2007) and used as a benchmark warming scenario by others (e.g. Dossena *et al*., 2012; Yvon-Durocher, *et al*., 2010)), resulted in the CTmax exceedance of 90% of primary consumers and 88% of secondary consumers.

Thermal stress was indicated by four taxa representing collectors, grazers, predators and shredders at water temperatures below CTmax (Table 5.4). Pre-CTmax phenotypes were expressed 0.5 °C (*Radix balthica*, grazer), 1.4 °C (*Gammarus pulex*, shredder), 2.5 °C (*Asellus aquaticus*, collector) and 13.1 °C (*Calopteryx virgo*, predator) prior to each taxon's mean CTmax.

Eighty-nine percent of taxa investigated (not including adult Elmidae) possessed either gill or tegument respiration (Figure 5.8). There was variability within respiratory mode groups, with, for example, 57% and 55% of taxa possessing gills and tegument respiration, respectively, having a CTmax lower than 31.1°C, whilst the remaining taxa exhibited a greater CTmax (Figure 5.8a-b). All taxa with either plastron or spiracle respiration had a CTmax greater than 31.1°C (Figure 5.8c-d), highlighting the importance of respiratory mode in determining thermal activity thresholds. There were no clear relationships between maximum potential size and CTmax (Figure 5.9), nor dispersal type (Figure 5.10) or number of generational cycles (Figure 5.11). For each of these traits, variability was great within modalities, and similar across modalities, suggesting the importance of other traits or 'trait-combinations' in influencing overall thermal tolerance.

Differences between CTmax and HC may be partly determined by mode of respiration. Typically, greatest differences between CTmax and HC were for taxa with tegument respiration. Conversely, taxa with spiracle or plastron respiration had smaller differences between CTmax and HC. There were exceptions to the pattern between respiratory mode and HC-CTmax difference, e.g. the greatest difference (9.3 °C) and smallest difference (0.6 °C) were both for taxa possessing gills.

Figure 5.6. **Mean ±1SE CTmax of macroinvertebrates grouped by functional feeding group**. Showing a) Collectors and b) Grazers. Horizontal dashed lines indicate maximum water temperature in flowing river (bottom) and stagnant pool (top). CTmax values below dashed lines illustrate potential loss of functioning.

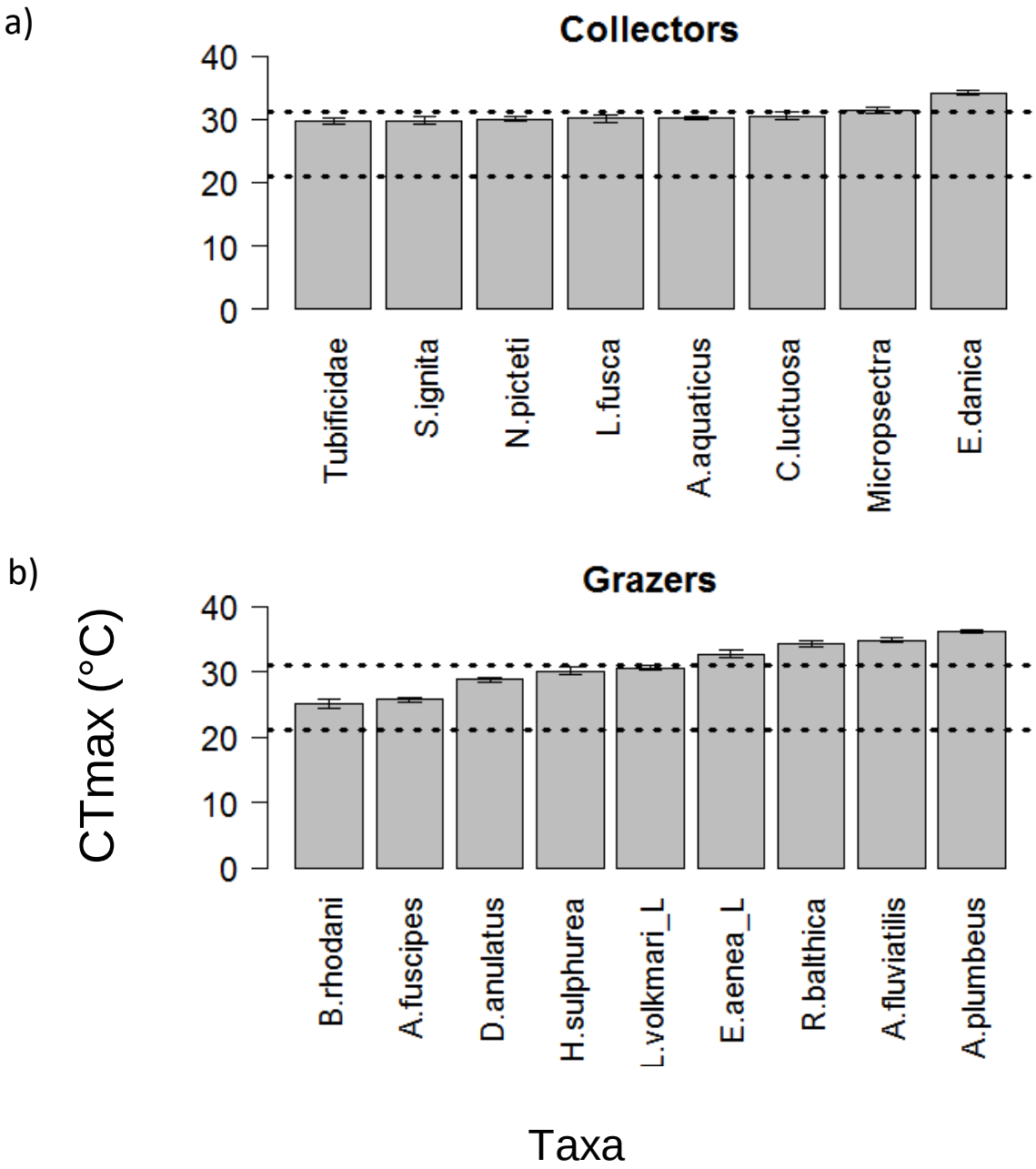


Figure 5.7. **Mean ±1SE CTmax of macroinvertebrates grouped by functional feeding group**. Showing a) Predators, b) Shredders and c) Filterers. Horizontal dashed lines indicate maximum water temperature in flowing river (bottom) and stagnant pool (top). CTmax values below dashed lines illustrate potential loss of functioning.

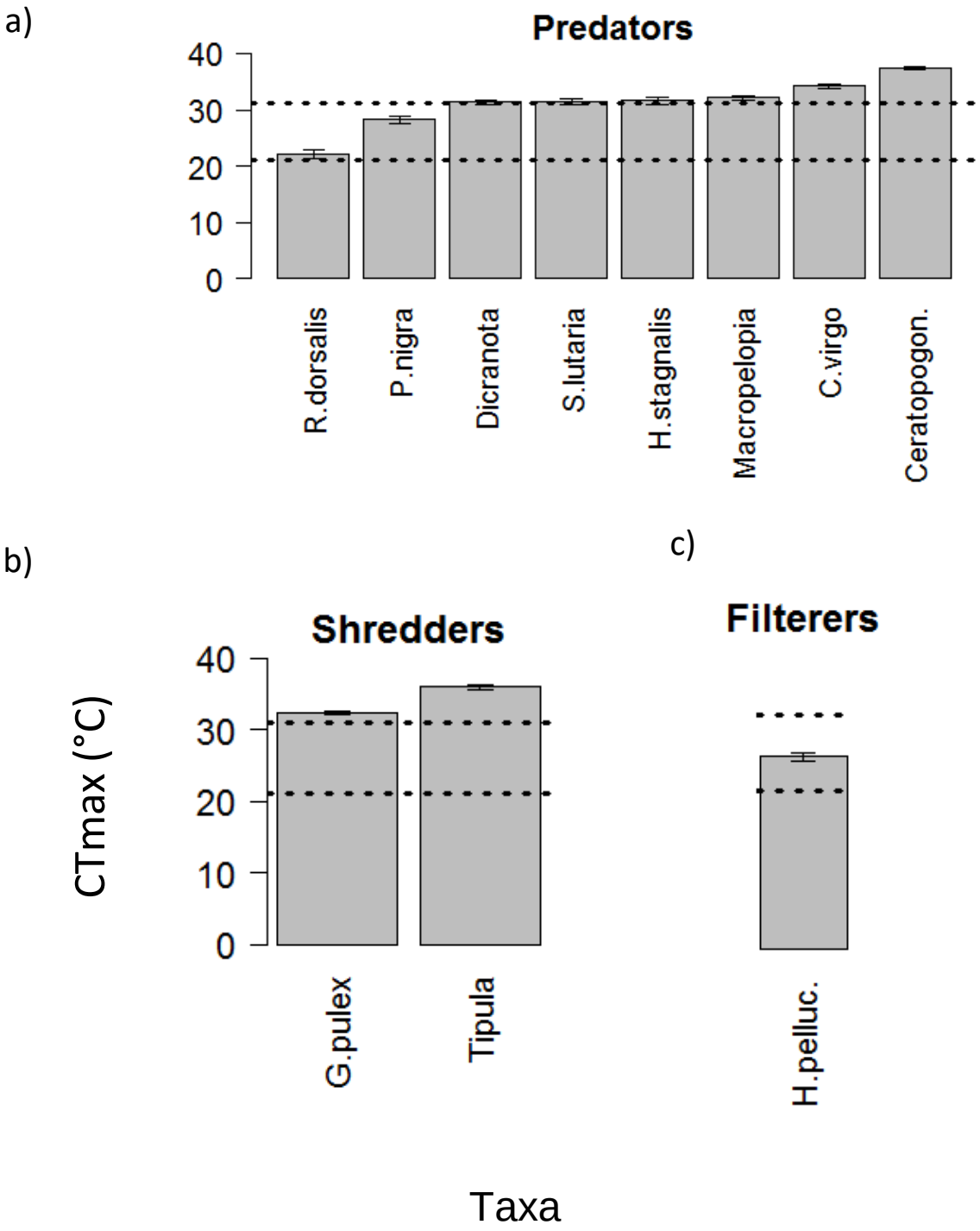


Figure 5.8. **Mean ±1SE CTmax of macroinvertebrates grouped by (main) mode of respiration**. Showing a) Gills, b) Tegument, c) Plastron and d) Spiracle. Horizontal dashed lines indicate maximum water temperature in flowing river (bottom) and stagnant pool (top). CTmax values below dashed lines illustrate potential loss of functioning.

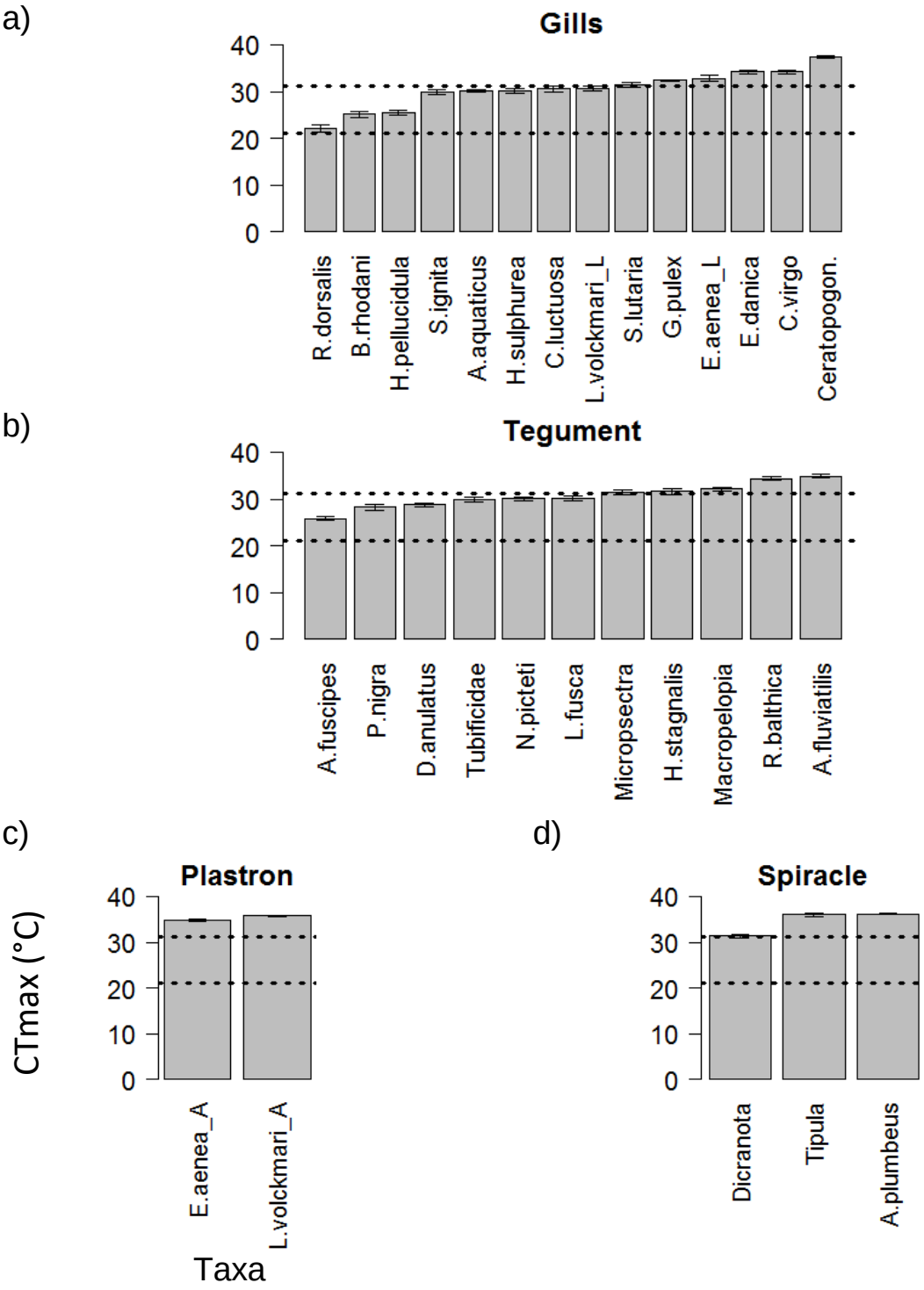


Figure 5.9. **Mean ±1SE CTmax of macroinvertebrates grouped by maximum potential size**. Showing a) >0.25-0.5 cm, b) >0.5-1 cm, c) >1-2 cm, d) >2-4 cm and e) 4-8 cm. Horizontal dashed lines indicate maximum water temperature in flowing river (bottom) and stagnant pool (top). CTmax values below dashed lines illustrate potential loss of functioning.

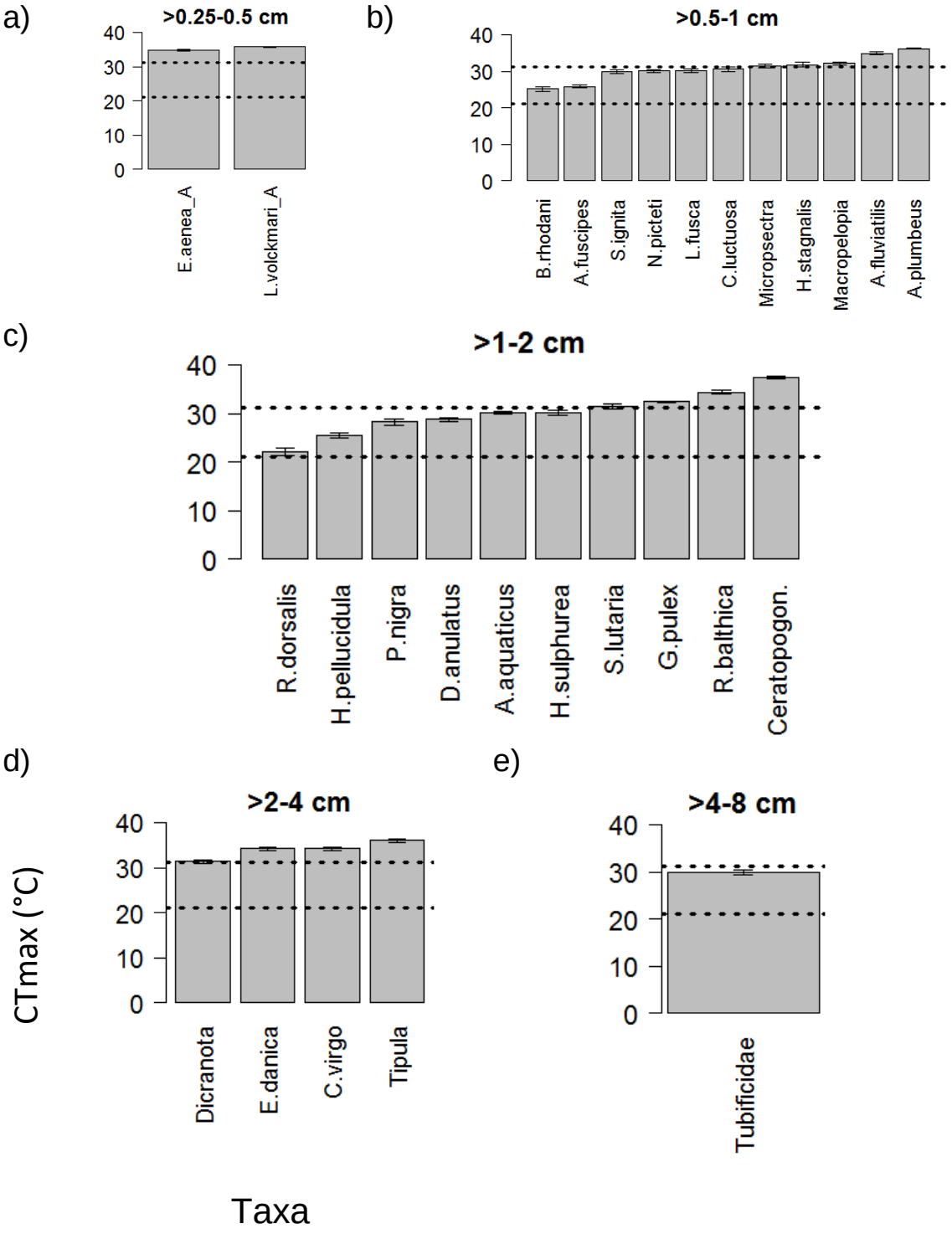
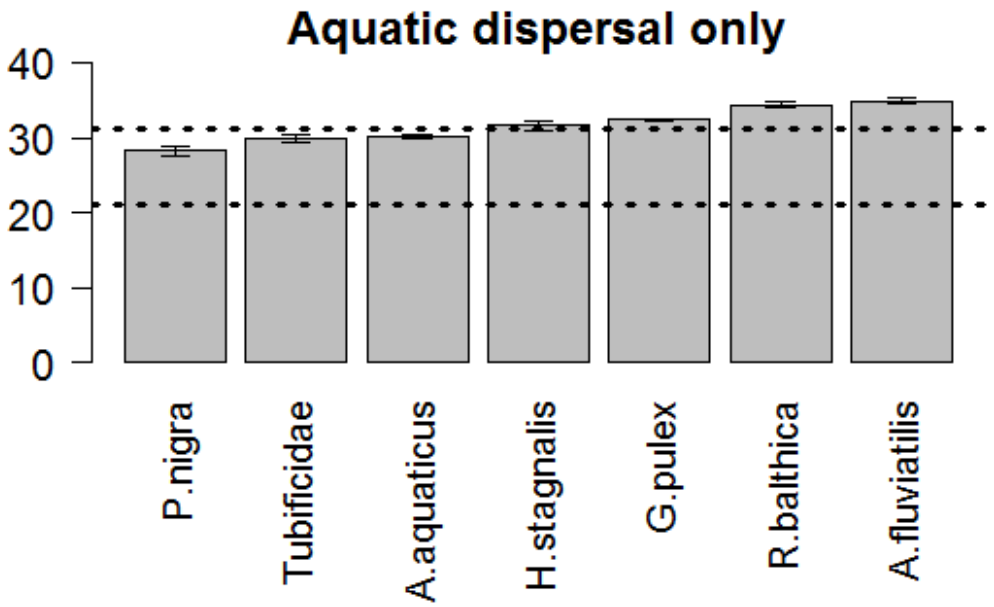


Figure 5.10. **Mean ±1SE CTmax of macroinvertebrates grouped by dispersal mechanism**. Showing a) Aquatic dispersal only and b) Aquatic and terrestrial dispersal. Horizontal dashed lines indicate maximum water temperature in flowing river (bottom) and stagnant pool (top). CTmax values below dashed lines illustrate potential loss of functioning.

a)



b)

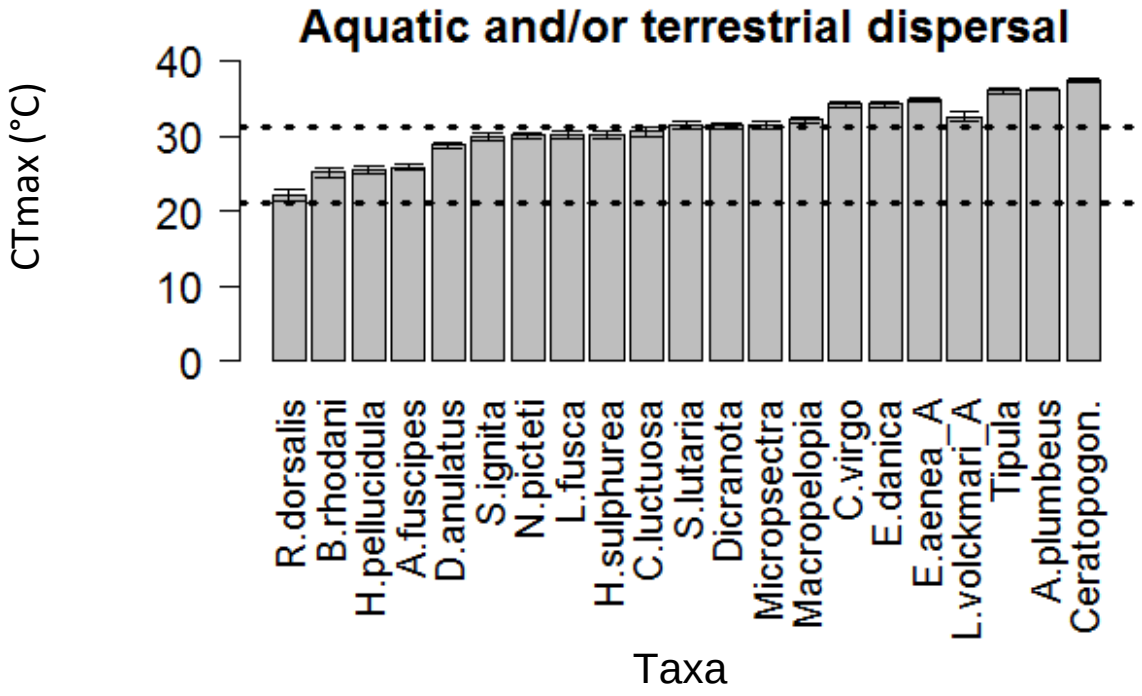
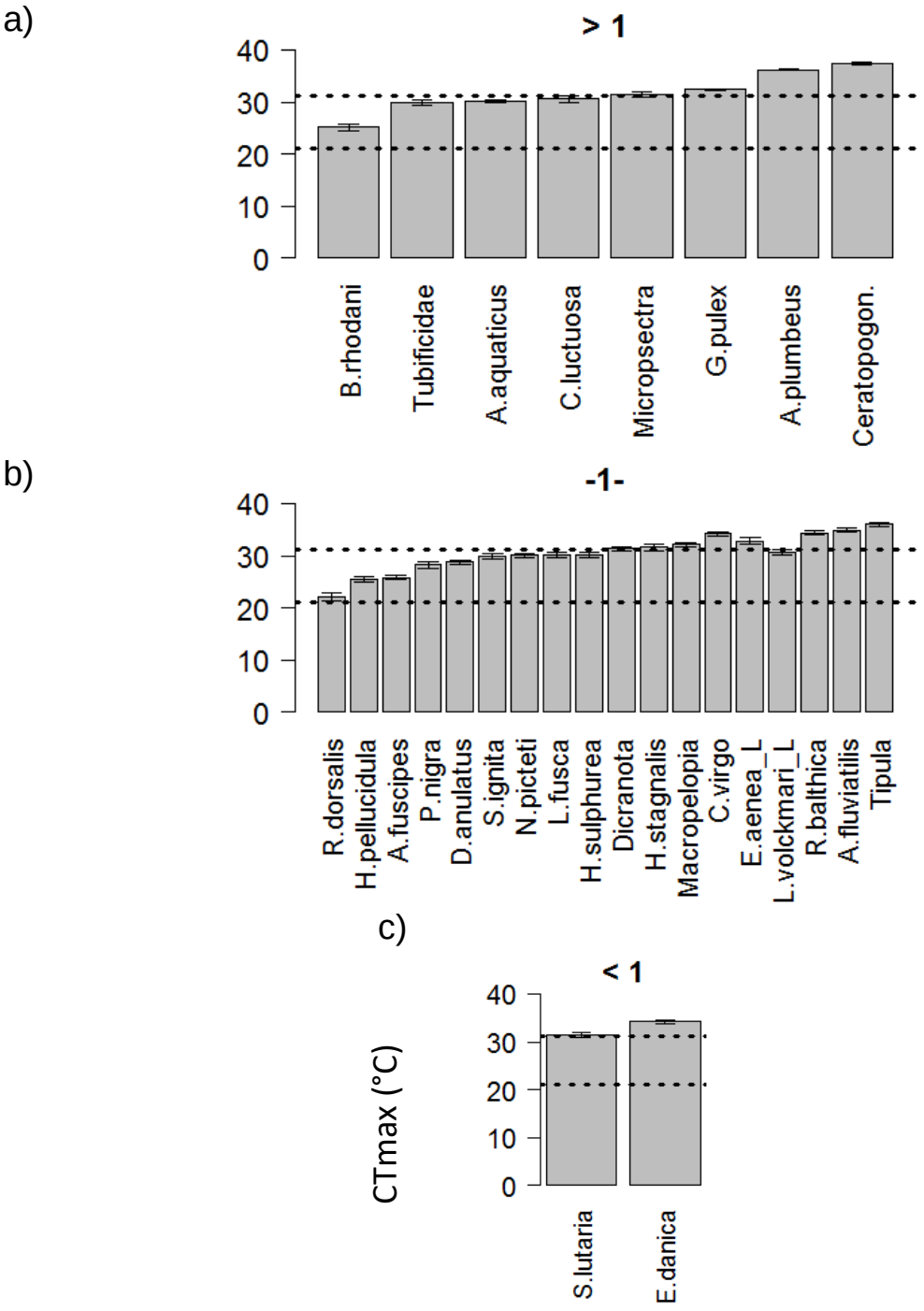


Figure 5.11. **Mean ±1SE CTmax of macroinvertebrates grouped by number of annual generational cycles**. Showing a) more than one, b) only one and c) less than one. Horizontal dashed lines indicate maximum water temperature in flowing river (bottom) and stagnant pool (top). CTmax values below dashed lines illustrate potential loss of functioning.



5.5 DISCUSSION

Physiological responses at the individual and population level may have ecological repercussions at higher levels of ecological complexity. This study investigated the warming tolerance of 28 chalk stream macroinvertebrates. The main findings from this chapter are 1) CTmax varies considerably between taxa suggesting that climate change and extreme events will result in clear winners and losers, 2) CTmax is a double edged sword as greater CTmax comes with a cost – a reduction in thermal plasticity, 3) Hydrological regime plays an important role in determining stream water temperature, with flow buffering against temperature extremes, 4) macroinvertebrate mediated functional processes are more likely to persist if stream flow is maintained during summer months (as this prevents temperatures exceeding the CTmax of the taxa) but processes may be compromised following flow cessation if water temperatures significantly increase, 5) phenotypes exhibited prior to CTmax may suggest a cessation of functioning at water temperatures that will more realistically be experienced by taxa in future in the U.K., and 6) respiratory mode influences thermal activity thresholds, with taxa possessing plastron and spiracle respiration typically exhibiting a greater CTmax, and a smaller difference between HC and CTmax, compared to taxa possessing other modes of respiration, such as tegument.

Effect of warming on functional feeding groups and processes.

Maximum water temperature recorded in a flowing stream (21.1 °C) did not exceed the CTmax of any of the 28 chalk stream macroinvertebrate taxa. On the other hand, maximum water temperature recorded in a stagnant pool (31.1 °C) exceeded the CTmax of 50% of taxa. Whilst stagnant isolated pool temperature was sufficient to

exceed the CTmax of all five functional feeding groups considered (collectors, filterers, grazers, predators and shredders), it did not 'eliminate' entire functional groups (excluding filterers for which only a single taxon was tested). Therefore, at maximum water temperatures recorded in a stagnant pool, a proportion of taxa representing different functional groups would persist, owing to physiological diversity among constituent FFG members. The impact this would have on functional processing rates is unknown, but would depend on a multitude of factors including functional redundancy (Jonsson *et al*., 2002), species identify (Wojdak & Mittelbach, 2007), and the importance of facilitative interactions between constituent members of each FFG (Cardinale *et al*., 2002; Costantini & Rossi, 2010; Jonsson & Malmqvist, 2000). If competition for resources within functional feeding groups is great, the temporary loss of functioning of a taxon may increase resource availability for other taxa which possess a greater CTmax, especially where taxa with a low CTmax dominate the community (Dangles & Malmqvist, 2004). In the long term, this could modify the dominance of taxa within the food web and allow taxa with lower competitive abilities to flourish. It should be noted here that the functional feeding group concept is not rigid, and plasticity can result in taxa falling into multiple functional feeding groups (e.g. Macneil *et al*., 1997). Therefore, although the dominant FFG modality was assigned to each taxon in this study, exceedance of CTmax would temporarily suspend other functions in addition to the main assigned function (e.g. exceedance of *G. pulex* CTmax would cease shredding, but probably also the collection of detritus, and predation).

Where CTmax of a taxon was less than maximum water temperature recorded, the functional provisioning of that taxon would undoubtedly cease. If water temperature

is restored to within a tolerable range in sufficient time, mortality may be avoided and normal functioning by the organism will resume (e.g. Beitinger *et al*., 2000; Coombs & Bale, 2014; Re *et al*., 2006; Diaz *et al*., 2011; Fangue & Bennett, 2003). Maximum water temperature of an isolated pool was sufficient to exceed the heat coma of two sensitive species; *R. dorsalis* and *B. rhodani*. As it is known that heat coma is very close to physiological death (Chown & Nicolson, 2004), it is likely that these sensitive taxa would be extirpated, but further work is required to confirm this. Therefore a loss of functioning in this instance would be longer term (McIntyre *et al*., 2007), and would reduce resilience and the rate of ecological recovery following a return to preferable water temperatures.

Activity threshold exceedances may have important effects on predator-prey feeding links. This could have the effect of altering energy flow pathways through the food web despite no occurrence of direct mortality. For example, the predatory leech *Helobdella stagnalis* is known to feed on *Radix balthica* (Martin, 1994; Young, 1980), whilst *R. balthica* is known to be a gregarious consumer of algal resources (O'Gorman *et al*., 2012). The activity threshold data obtained would suggest that at temperatures > 31.6 °C but < 34.3 °C, the feeding link between *H. stagnalis* and *R. balthica* would be broken owing to the exceedance of *H. stagnalis* CTmax, despite both taxa remaining present. This may release *R. balthica* from predation (not considering other predators that may be present), increasing algal grazing pressure. At temperatures > 40 °C but < 40.8 °C, despite *H. stagnalis* exceeding HC, grazing pressure may again be reduced owing to the exceedance of *R. balthica* CTmax. The effect on predator-prey interactions will however depend largely on the ecology of remnant taxa. For example, sit-and-wait predators, which rely on movement to

induce attacks, may be negatively affected by prey immobility in situations where prey have a lower CTmax than their predators, as the encounter rate will be reduced leading to fewer attacks (Dell *et al*., 2014). On the other hand, searching predators may be able to maintain sufficient encounters with prey by increasing foraging efforts.

Despite the importance placed on CTmax as a physiological threshold to warming, other phenotypes were recorded for four taxa – representing each of collectors, grazers, predators and shredders – prior to CTmax that may signify thermal stress and a loss of functioning. For example, both *A. aquaticus* and *R. balthica* were unable to grip vertical surfaces prior to reaching complete locomotory control, resulting in movement constrained to the horizontal surface of the test arena. *G. pulex*, became unable to utilise the three dimensional space of the water medium to move, and so was too constrained to the arena floor. *Calopteryx virgo* showed signs of distress early on during the temperature ramp. In the case of *G. pulex* and *A. aquaticus*, an early onset of central nervous system dysfunction may explain observed pre-CTmax phenotypes (Hazell & Bale, 2011) whilst the phenotype observed by *C. virgo* may be a behavioural mechanism in an attempt to reduce the boundary layer between the lamellae and surrounding water (Verberk & Calosi, 2012). The mechanisms underpinning phenotypes prior to CTmax may not be well understood, yet it is probable that such threshold exceedances will impair functional processes. For example, it would prove most challenging for *R. balthica* to graze algae from surfaces such as pebbles and cobbles when restricted only to horizontal surfaces.

Warming and activity thresholds

CTmax was highly variable among taxa and ranged from 22.0 °C to 37.3 °C. When ranked by CTmax in ascending order, with the exception of *Ephemera danica,* all EPT taxa were within the 13 most sensitive taxa, whilst all Dipterans were within the 15 least sensitive taxa. In particular, Trichopterans as an Order were the most sensitive of the 28 taxa investigated. Similar trends across taxonomic groups were found by Gaufin & Hern (1971) with an Ephemeroptera and a Diptera taxon having the smallest and greatest thermal sensitivity, respectively. EPT taxa were highly sensitive (top 44%) in a study on South African macroinvertebrates by Dallas & Rivers-Moore (2012). Additionally, Ephemeroptera had lower activity thresholds than other taxa (Mollusca) in a study of New Zealand macroinvertebrates (Cox & Rutherford, 2000). Dipterans which typically (though not always) inhabit shallow and stagnant pools may have a greater CTmax as they experience a greater magnitude of temperature variation relative to some other orders (Deutsch *et al*., 2008; Sunday *et al*., 2011). *Polycelis nigra* exhibited a low activity threshold to warming supporting the notion that some cool water adapted Triclads can be particularly sensitive to warming (Durance & Ormerod, 2010). Activity threshold plasticity varied between taxa, with those possessing the greatest CTmax having the lowest variance, in support of the 'trade-off hypothesis' (Gunderson & Stillman, 2015). Such trade-offs are believed to be common, yet may not necessarily have an adaptive advantage (Pörtner *et al.*, 2006).

Whilst it has been possible here to make limited comparisons with other studies, evaluation of these results with others is challenging as studies typically only investigate a single species (e.g. Buchanan *et al*., 1988; Cottin *et al*., 2012;

Lagerspetz & Bowler, 1993) or taxonomic group (e.g. Moulton *et al*., 1993; Renault *et al*., 2005). As activity threshold values such as CTmax are, to a degree, an artefact of experimental procedures (i.e. influenced by choice of acclimation temperature and rate of warming; Chown *et al*., 2009), the results of one or a small group of taxa provide little scope for comparison across studies (Houghton *et al*., 2014). On the other hand, large datasets such as Dallas & Rivers-Moore (2012), as well as data collected in this chapter, allow meaningful taxonomic comparisons both within and across studies, yet remain scarce. Large datasets are important because activity threshold variation can often be partitioned at taxonomic levels which can be compared between studies (Chown, 2001).

Differences in heat coma ranged from 24.6 °C (*Rhyacophila dorsalis*) to 40.8 °C (*Ceratopogonidae* sp.), illustrating that HC activity thresholds were also highly variable among taxa. Along with *Ceratopogonidae* sp., both *Sialis lutaria* and *Radix balthica* exhibited the greatest heat coma values. *Ceratopogonidae* sp. and *Sialis lutaria* were two of only three taxa that persisted in drying pools throughout the duration of a study by Verdonschot *et al*. (2015), suggesting heat coma is highly correlated to, and sits closely to, physiological death. The difference between CTmax and HC varied from as little as 0.6 °C (*Baetis rhodani*) to 9.4 °C (*Agapetus fuscipes*). This demonstrates that whilst CTmax has previously been used as a measure of 'thermal tolerance' (Dallas & Rivers-Moore, 2012), this term should be used with caution, as CTmax was not always a good indicator of likely survival (i.e. HC, in the case of taxa such as *A. fuscipes*). Following a similar pattern to CTmax, EPT taxa generally had a low HC whilst Dipterans were among the taxa with the greatest HC. Taxa with a low CTmax and low HC are at greater risk of extirpation

following loss of locomotory control than those with a greater difference between CTmax and HC. Respiratory mode appeared to partly explain the variability in discrepancy between CTmax and HC, suggesting that oxygen regulation may ultimately determine this difference. Taxa possessing less efficient respiratory modes (e.g. tegument) exhibiting larger differences between CTmax and HC, may have difficulties in maintaining oxygen uptake at lower, yet elevated, temperatures, despite being physically able to reach much greater temperatures prior to physical damage occurring. Conversely, taxa with more efficient respiratory mechanisms (e.g. spiracle) are more likely to be able to 'resist' CTmax for longer, owing to sufficient oxygen uptake to meet metabolic demands, until closely approaching temperatures that inflict physical damage such as protein denaturation.

Although there is some agreement that, for example, certain taxonomic groups are more or less sensitive than others irrespective of temporal and spatial differences, the mechanisms underlying such variability (both within and across species) is still not well understood. Whilst beyond the scope of this chapter a few potential mechanisms are considered here. Dissolved oxygen solubility is inversely related to temperature, whilst warming increases metabolism, with both factors increasing DO demand (Verberk *et al*., 2011) and invoking an 'oxygen squeeze' whereby metabolic demands cannot be met (Ficke *et al*., 2007). This may result in oxygen limitation and asphyxiation for aquatic macroinvertebrates (Verberk & Bilton, 2011), reducing the ability of taxa to maintain sufficient uptake (Puckett & Cook, 2004). Mode of respiration may greatly affect activity thresholds of aquatic (Chessman, 2015; Verberk & Bilton, 2013) and terrestrial (Lighton, 2007) organisms, and often the most and least thermally sensitive taxa possess contrasting modes of

respiration (Tachet *et al*., 2010). Findings from the current study indicate that air breathers and taxa possessing plastron respiration (and to a lesser degree taxa possessing gills) (e.g. *Anopheles plumbeus*, *Ceratopogonidae* sp., Dicranota sp. and *Tipula* sp.) may be better at regulating oxygen at elevated temperatures.

Oxygen deprivation is believed to drive thermal activity thresholds before the onset of other mechanisms such as protein function loss (Portner, 2001). However, others argue that oxygen delivery beyond CTmax may be sufficient to maintain aerobic metabolism, implying that additional mechanisms are responsible for determining taxa activity thresholds (Mölich *et al*., 2012). A detailed discussion is not provided here as in-depth reviews have been provided by others (Chown & Terblanche, 2006). Differences in the ability of taxa to withstand membrane permeability alteration (Koopman *et al*., 2016) and protein denaturation (Somero, 2003) at elevated temperatures may account for the observed variability in activity thresholds such as HC, with some arguing that thermal tolerance is genetically determined (DeKozlowski & Bunting II, 1981). This may in part be regulated by heat shock protein (Hsp) response, in particular Hsp70, (Nielsen *et al*., 2005) which bind to denaturing proteins in response to temperature extremes, and repair them (Feder & Hofmann, 1999). Thermal stress that induces Hsp response in aquatic systems will most frequently occur in organisms inhabiting shallow, stagnant, warmer waters (Feder & Hofmann, 1999; Kelley *et al*., 2011), although Hsp expression may also vary among individuals of the same population owing to other factors such as ontogeny (Arias *et al*., 2011) which may account for some variability in activity thresholds (Chown & Gaston, 1999). For example, differences in body size between individuals of the same species can determine Hsp response, within smaller

gammarids exhibiting a weaker response in a study by Grabner *et al*. (2014). Therefore body size may indirectly influence thermal sensitivity mediated via Hsp response. Other sources of variability may have included digestive status (although all housed taxa were starved) and age, with an age difference of as little as 14 days significantly affecting thermal sensitivity of fruit flies in a study by Nyamukondiwa & Terblanche (2009).

The study demonstrates that oxygen must play a critical role in determining thermal activity thresholds such as CTmax, and therefore respiratory mode may lead to winners and losers when oxygen supply is limited during warming. In particular, spiracle respiration resulted in higher CTmax values, reflecting a greater ability to maintain oxygen demand via aerial exchange (Verberk *et al*., 2016) relative to taxa relying on dissolved oxygen, which can become limiting. The importance of respiratory mode is too reflected in CTmax differences throughout the life cycle of Elmidae, which predominantly use gill respiration during their larval form and plastron respiration in their adult form. This resulted in greater CTmax values of adults, relative to larvae, highlighting the greater efficiency of plastron respiratory mode, relative to gills. In addition to respiratory mode, taxa were grouped by maximum potential body size to investigate the effect of size modalities on thermal activity thresholds. Although body size can influence Hsp response, there was no obvious correlation between maximum potential body size and thermal activity thresholds. Body size however may influence thermal activity thresholds in other ways, for example by determining metabolic demand (Gillooly *et al*., 2001) which again links to oxygen supply and demand. Furthermore, body size relates to surface area, which has implications for desiccation resistance during warming (Oberg *et*

al., 2012) as well as again influencing respiration of taxa possessing particular respiratory modes such as tegument. Dispersal type may influence thermal activity thresholds through differential exposure to elevated, sub-lethal temperatures (van Dooremalen *et al*., 2013). For example, taxa able to disperse easily within aquatic systems may be better able to switch between microclimates as environmental conditions change, whilst those with poor dispersal abilities will be subjected to unfavourable temperatures attributable to natural environmental fluctuations. In this study, *P. nigra* and *A. fluviatilis* exhibited the lowest and greatest CTmax of the aquatic-only dispersers, respectively. *Polycelis nigra* is capable of dispersing at a greater rate relative to *A. fluviatilis*, and this may provide evidence to suggest that the most immobile taxa are subjected to greater temperature fluctuations, and via acclimation, are able to tolerate greater elevated temperatures. Although some taxa capable of aerial dispersal can escape warmed waters in summer, leading to a reduction in exposure to elevated yet sub-lethal temperatures (Larned *et al*., 2010), taxa possessing aerial dispersal capabilities exhibited some of the greatest CTmax values in the present study (e.g. *Ceratopogonidae* sp., *A. plumbeus*, *Tipula* sp.. Further work is needed to determine the importance of dispersal capabilities on the thermal activity thresholds of macroinvertebrates, as the scope of this study only permits speculative conclusions to be drawn. Typically '*r*-selected' taxa are able to rapidly colonise areas that experience disturbances which lead to the loss of other taxa (Chiu & Kuo, 2012), and so may have a greater tolerance towards elevated temperatures. In the current study, the number of generational cycles per year were investigated as a surrogate for *r*-selected taxa (multiple cycles per year = multivoltine). However, no clear pattern was found between the number of

generational cycles of taxa and their CTmax, and thus further work is required to determine the physiological mechanisms that underpin the success of *r*-selected taxa. A lack of obvious patterns between any of the traits 'maximum potential body size', 'number of generational cycles' and 'dispersal type' and thermal activity thresholds such as CTmax suggest that no one of these traits is of ultimate importance. It is thought that respiratory mode is of the greatest importance in determining CTmax, with other traits acting in-combination to determine overall thermal tolerance. In trait analyses conducted elsewhere, mode of respiration and temperature preference have been shown to correlate with drought tolerance, with plastron and spiracle respiration and thermophily corresponding with increased resistance (Chessman, 2015; Díaz *et al*., 2007).

Effect of hydrological regime on water temperature

Water temperature approached but never exceeded the CTmax of the most sensitive taxa in flowing lowland streams. Streamflow buffered against extremes in surface air temperatures, with maximum temperatures varying from 17.6 °C to 21.1 °C. This helped increase a suitable distance between the CTmax of many taxa and maximum water temperature. However, a mean increase of +4 °C by the end of the century (IPCC, 2013) may raise summer water temperatures beyond the activity thresholds of sensitive species (Durance & Ormerod, 2010) whose CTmax were found to be close to maximum water temperature (e.g. *R. dorsalis*). It remains to be explored whether prolonged exposure (relative to the experimental warming rate used) to temperatures below CTmax may have physiological implications which

may result in a lowered CTmax. The extent to which oxygen deprivation determines activity thresholds may largely influence the effect of exposure times to elevated temperature. Stagnation resulted in a maximum water temperature of 31.1 °C, exceeding the CTmax (50%) and HC (7%) of the 28 taxa investigated. Evaporative cooling reduces the rate of warming in water at temperatures beyond 20 – 25 °C (Bogan e*t al*., 2006; Mohseni *et al*., 1999; Mohseni *et al*., 2003), and plays a significant contribution to the heat energy budget in U.K. lowland streams (Webb & Zhang, 1999) but was insufficient to prevent a shallow and stagnant pool from exceeding the CTmax of many taxa in this research.

An extensive search of the scientific literature revealed a shortfall of studies that report extreme water temperatures in streams and rivers. Two studies were found which investigated fish mortality in shrinking pools, with an isolated pool in Ohio, U.S.A., 1975, reaching 32 °C (Tramer, 1977) whilst 39.5 °C was reached in an unshaded pool in a different Ohio river, U.S.A., in 1988 (Mundahl, 1990). River water temperature exceeded 40 °C in an Oklahoma stream, U.S.A. during extreme low flow in 2000 (Galbraith *et al*., 2010), when water depth fell to below 2 cm. Pool water temperatures in a New Zealand river in 2011 also exceeded 40 °C following flow cessation, recorded when pool depth approached 0 mm from the pool bottom (Drummond *et al*., 2015). The authors in this latter study highlight how environmental values such as pH, electrical conductivity, turbidity and dissolved oxygen fluctuate and confound temperature as isolated pools shrink, but yet we know very little about how such stressors may interact with temperature to reduce activity thresholds such as CTmax. However, by studying activity thresholds and water temperatures independently within the current study, functional vulnerability

of taxa to warming can be directly determined, and it is quite certain that warming alone during extreme compound events will result in reduced taxa functionality and increased mortality, though more work is needed to disentangle dissolved oxygen and temperature (Verberk & Calosi, 2012) as well as short and long term warming effects (Nyamukondiwa & Terblanche, 2010).

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The method used in this study is a standard technique to rapidly assess the thermal tolerance of macroinvertebrates. Method variables were also consistent with previous studies (e.g. rate of warming). However it could be argued that sustained warming at the rate used is not realistic of natural environments. On the other hand, lower rates of warming can develop their own limitations, such as increased exposure of test subjects to elevated temperatures. Moreover, the choice of acclimation temperature used is context dependent to specific studies, and as such the dataset produced from this experiment may not be directly comparable to other studies that may use different parameter values. When comparing between studies, it is imperative that method variables are checked first to determine the ease of comparability. A further limitation to the study is that few readily accessible datasets contain recordings of water temperature during periods of extreme flow (i.e. fixed gauging loggers are exposed to air), and as such the river water temperature time-series dataset used to compare against taxa thermal activity thresholds was limited. Care was taken to ensure logging methodologies were approximately consistent. However, comparison between macroinvertebrate CTmax values and experimental and natural river water temperatures are limited, until further extreme river water temperature outputs from other studies come to light.

5.6 CONCLUSION

This chapter reveals that the warming tolerance of lowland chalk stream macroinvertebrates is reduced when streams cease flowing and water temperature is elevated. Stagnation reduced the gap between water temperature and CTmax, and in many cases water temperature exceeded activity thresholds such as CTmax and heat coma. Stream flow therefore buffers water temperatures from extremes for even the most sensitive taxa investigated, but future climate will probably have deleterious effects on stream functioning via physiological mechanisms mediated by rising temperatures. A lack of activity threshold studies spanning large numbers of taxa are limited, as are studies that investigate water temperature extremes, and it is therefore challenging to make comparisons between studies across both space and time, and to infer warming tolerances of taxa. A central challenge now for physiologists and ecologists alike is to understand how warming during extreme events such as drought may interact with other stressors to influence the physiological responses of macroinvertebrate taxa. Moreover, further trait analyses incorporating measurements of CTmax and HC are needed, to be able to better understand the mechanisms which underpin thermal activity thresholds, and to confidently predict severe, future warming effects on aquatic communities.

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CHAPTER SIX

General discussion

6.1. UTILITY OF EXPERIMENTS IN DROUGHT-STRESSOR RESEARCH

This research project has quantified ecological responses to drought stressors at the autecological, synecological and ecosystem level. By taking an experimental approach it has been possible to identify causal mechanisms that underpin drought ecological responses, providing insights into the importance of individual stressors at multiple levels of ecological complexity. Furthermore, laboratory and field experiments allowed effects to be quantified from the level of the individual to the whole ecosystem. To test for the effects of reduced flow on ecological responses, manipulative experiments are clearly required to overcome confounding issues faced by aquatic ecologists (Bunn & Arthington, 2002). These findings provide insight which can inform water management and conservation decisions in future.

When stressors co-occur during natural drought events, it proves extremely challenging to disentangle causal mechanisms of drought effects due to the confounding nature of water loss that coincides with other extraneous pressures. From empirical observations we therefore may know *what* the effects of drought are, but knowledge of *how* and *why* such effects occur are not so apparent. This requires careful, controlled and manipulative experimental execution. This research has combined laboratory and field experiments to help decipher the mechanisms behind ecological responses.

6.1.1 DROUGHT STRESSORS AS CAUSAL MECHANISMS

The first overarching aim of this research was to "*determine the underpinning mechanistic basis of hydrological drought effects*". This was achieved by: studying warming effects, independently, on macroinvertebrate physiological thresholds;

studying sedimentation effects, independently, on predatory consumption rates; and studying independent and in-combination effects of warming, sedimentation and dewatering on a range of ecological and functional receptors. Dewatering associated with hydrologic drought reduces the thermal capacity (Hannah & Garner, 2015; Webb & Nobilis, 2007) and increases residency (Mosley, 2015; van Vliet & Zwolsman, 2008) of the water body, elevating temperatures beyond typical maxima of running water (Verdonschot *et al*., 2015). This was observed in Chapter 5 whereby water temperature of an isolated pool greatly exceeded that of the running waters investigated. Warming effects on macroinvertebrate individuals were found to be variable among taxa, as evidenced by Critical Thermal Maximum (CTmax) and Heat Coma (HC) phenotypes in Chapter 5 and in agreement with similar studies (e.g. Dallas & Rivers-Moore, 2012), highlighting the need to better understand physiological thresholds to predict taxa responses to thermal stress (Dallas, 2008). By achieving the three objectives in Chapter 5 (assess thermal activity thresholds of macroinvertebrates; assess lowland river water temperatures; and compare activity thresholds with water temperatures) it was possible to determine the 'warming tolerance' of key macroinvertebrate taxa to natural water temperatures. This contributed to the first primary overarching aim of the research; the response of taxa to thermal pressures may underpin higher ecological responses to drought such as altered community composition and functional processing rates. It is believed that the variability in taxa physiological thresholds observed in Chapter 5 (i.e. CTmax range = 15.3 °C HC range = 16.2 °C) is a fundamental mechanism underpinning idiosyncratic species losses to drought that are commonly reported in the wider literature (e.g. Lancaster & Ledger, 2015). For example, the taxon with

the greatest HC (*Ceratopogonidae* sp.; HC = 40.8 °C) was one of only three taxa to persist during streambed desiccation in a study by Verdonschot *et al*. (2015), whilst the taxa exhibiting the lowest values all belong to the EPT orders and are known to be particularly sensitive to drought (Calapez *et al.*, 2014). These findings therefore advance the field of disturbance ecology by developing our understanding of causal mechanisms underpinning drought ecological responses, which are otherwise largely unknown. A further physiological advancement of Chapter 5 was the finding that respiratory mode may partly determine CTmax. Taxa possessing spiracle and plastron modes of respiration were mostly found to exhibit greater thermal activity thresholds than other respiratory modes. Therefore, not only have the mechanisms been explored that determine ecological responses to drought, but so too have the mechanisms that may underpin the physiological response of the taxa, thereby cementing the link between physiology and aquatic ecology that has to date been challenging to do (Gaston, 2009).

Enhanced predator foraging efficiency, as evidenced in Chapter 4, illustrates heightened predator-prey encounter rates in response to habitat simplification (Hagen et al., 2012; Hossie & Murray, 2010; Manatunge *et al*., 2000) and exemplifies indirect biotic mechanisms that regulate population size during extreme events. Attack rate and prey consumption increased with sedimentation, as predicted by the hypotheses outlined within Chapter 4, in line with similar studies elsewhere (e.g. Alexander *et al*., 2015). Knowledge of altered biotic interactions as forcing factors contributing to drought ecological response is exceedingly sparse and often only speculated to be a controlling mechanism on community structure (e.g. Dollar *et al*., 2003). This research therefore provides quantifiable evidence of

modified biotic interactions under conditions typical of drought. Furthermore, the findings suggest that benthic fish predators such as bullhead are probably far from satiation under normal stream conditions (Woodward & Hildrew, 2002) and when given the opportunity during drought will greatly increase total population proportional mortality, with up to ~75 individuals of *Gammarus pulex* consumed by *Cottus gobio* within a 24 hour period (Chapter 4). This research thus betters understanding of drought ecological impact causal mechanisms as set out in Chapter 1, evidencing that biotic impacts are not a simple cause-and-effect relationship between abiotic stress and taxa, but are too driven by indirect effects, mediated through the food web. This has implications on stream resilience, as strengthened biotic effects may increase top-down control, exacerbating abiotic drought effects and hampering stream recovery success following the return of flow. A key finding of Chapter 4 was also the discovery of an interaction between substrate and prey density, whereby greatest proportional prey consumption occurred when low prey densities and sediment addition were combined. The ability of macroinvertebrates to mobilise and congregate in pools during drought as has been demonstrated elsewhere (e.g. Covich *et al*., 2003), along with the degree of sedimentation prior to streambed fragmentation will thus determine the extent of proportional prey consumption by stream predators.

The study of main and in-combination effects in Chapters 2 and 3 betters understanding of ecological responses to compound stress, increasingly becoming the norm in aquatic systems as the climate changes (Dudgeon *et al*., 2006; Strayer & Dudgeon, 2010). Compound stress was important in explaining community variation in outdoor mesocosms, with only treatments containing 2+ stressors (WS,

SD, and WSD) significantly explaining total community variation in pairwise RDA comparisons. Interactions between stressors triggered significant ecological effects (community structure and ecosystem functioning) when stressors were combined (three way ANOVA; Chapters 2 and 3), as has been reported elsewhere in similar studies with differing contexts (e.g. Matthaei *et al*., 2010; Piggott *et al*., 2015; Wagenhoff *et al*., 2012). Occasionally, these interactions appeared to be facilitative and synergistic, again highlighting the importance and deleterious nature of compound stress in determining ecological response (Brook *et al*., 2008). A complex interaction between sediment, warming and dewatering in Chapter 3 explained differences in *Berula erecta* growth rate between treatments: it was found that the level of dewatering (applied, not applied) influenced a two-way interaction between warming and sediment. This reinforces the notion that stressors can interact in complex ways to elicit effects that cannot be simply predicted additively, and reiterates the importance of manipulative experiments to better understanding of drought stressor interactions. Drought stressors also invoked main effects where the direction and magnitude of effect was similar with or without the presence of additional stressors. In chapter 2, the direction of such effects varied for each taxon, believed to account for the lack of total density main effects observed. Taxon density vectors were frequently orientated away from sediment treatments, demonstrating the overall deleterious nature of this stressor. Some taxon vectors however were positively correlated with sediment (i.e. *Micropsectra* sp.), demonstrating ecological winners during drought. Warming frequently interacted with additional stressors to determine macroinvertebrate community structure in Chapter 2, corresponding with findings from other stressor interaction studies (e.g. Piggott *et al*., 2015). The

mesocosm experiment was thus a useful tool to determine the importance of individual drought stressors, and the importance of interactions when these stressors were in-combination, and has provided building blocks for further research to investigate both additional stressors and differing levels of stress magnitude.

6.1.2. DROUGHT STRESSORS ACROSS MULTIPLE ECOLOGICAL LEVELS

The second main overarching aim of this research was to "*determine if and how drought pressures lead to effects at multiple levels of ecological complexity*". This was achieved by investigating ecological receptors from the individual (thermal activity thresholds and predatory impact) to macroinvertebrate populations and communities, and from small patch-scale descriptors (macroinvertebrate biomass standing stock) to production (e.g. macrophytre relative growth rate) to whole-system metabolism, resulting in the piecing of multiple hierarchical ecological levels within and across the thesis chapters. The deleterious effects of drought were evident across all levels of ecological response examined: Individual level responses included physiological tolerances to warming (Chapter 5), and behavioural mechanisms to dewatering and sedimentation (Chapter 4). Both of these findings highlight how effects at the individual level of a species may determine population level responses, supporting the notion that individual and population effects of different species are inextricably linked (Savage *et al*., 2004). It has recently been identified that research linking the effects of disturbances at multiple ecological levels is in its infancy, prompting the development of frameworks to determine environmental impacts of extreme events, by scaling effects from the individual to the ecosystem (Woodward *et al*., 2016). During extreme warming, the cessation of higher functioning and extirpation of populations is not random

(Jonsson *et al*., 2002), but rather, physiological responses at the individual determine functional impairment and mortality of taxa at higher levels of ecological complexity (Hunsicker *et al*., 2011). Predator foraging efficiency of prey individuals will also affect whole populations, as well as functional processes that are governed by prey taxa. The effect of habitat modification on searching predators may determine the time until prey extinction (Murdoch & Scott, 1984), whilst the effect on sit-and-wait predators may determine overall population stability (Hossie & Murray, 2010).

Flow cessation is a critical threshold that eliminates flow sensitive, rheophilic taxa such as *Hydropsyche* spp., *Rhyacophila* spp. and *Heptagenia* spp. (Calapez *et al*., 2014; Warfe *et al*., 2014) and flow cessation alone will reduce the size of the original stream food web (Ledger *et al*., 2013). The remnant community in resultant lentic pools is thus a resistant subset of the original community (Drummond *et al*., 2015), and stress applied in this research was insufficient to extirpate these remnant taxa (Chapter 2). Supporting the drought resistance hypothesis (Boersma *et al*., 2014), it is likely richness will persist among remnant macroinvertebrate taxa during drought until complete desiccation of the stream bed is achieved, highlighting the stepped, sequential nature of drought events (Boulton, 2003).

Common species with disproportionately important functional roles such as *Gammarus pulex* were greatly affected by drought stressors in Chapter 2, suggesting emigration/mortality in response to stress (Drummond *et al*., 2015), as well as possible intensification of biotic interactions and reduction of resources (Lake, 2003). Despite deleterious effects at the population level, climate change and disturbance events often lead to winners among taxa as well as losers (Somero,

2010), with *r*-selected taxa possessing rapid multivoltine life cycles filling the vacated niches of extirpated taxa (Ledger *et al*., 2011). This was observed in Chapter 2, with large densities of *Micropsectra* sp. appearing in warmed channels with added sediment. The magnitude of population change of dominant taxa is reflected in total macroinvertebrate biomass, illustrating the link between different ecological levels of complexity. Lentic taxa may also take the opportunity of flow cessation to infiltrate stagnant waters (Bogan *et al*., 2015), balancing transient taxa losses. In this case, richness is regulated by immigrant taxa, with turnover modifying the composition of biotic assemblages (Stewart *et al*., 2013). Without flow, and with terrestrial barriers between isolated pools impeding movement of aquatic biota, it is likely such effects are apparent only over temporal scales that are beyond the experimental duration of this research.

Patterns at the population level can too determine community responses; for example total density in Chapter 2 was driven solely by changes to taxa densities and never a result of changes to richness or community composition. Such effects have also been found elsewhere (Dewson *et al.*, 2007; Hille *et al*., 2014; Woodward *et al.*, 2015), suggesting community effects may commonly be the result of taxa population density changes. Moreover, it was found that differences in population densities in Chapter 2 resonated to differences in biomass of functional feeding groups in Chapter 3. For example, greater densities of large bodied gastropods such as *Radix balthica* (also mirrored by greater total densities) was evidenced by a larger grazer biomass; whilst fewer individuals of large bodied amphipods such as *Gammarus pulex* was reflected in a reduced shredder biomass. Thus, population effects have the capacity to indirectly alter processing rates at the functional level,

if such effects resonate to alter the biomass of key taxa (Chadwick & Huryn, 2005). However, changes to shredder biomass were not reflected in the rate of leaf litter decomposition in Chapter 3. Conversely, a reduction in shredder biomass reduced leaf litter decomposition elsewhere (Domingos *et al*., 2014; Martínez *et al*., 2013) prompting further work to investigate the link between FFG biomass and functional processes. Primary producers are integral in ecosystem functioning processes, and were found to be particularly sensitive to drought stressors in Chapter 3, in line with findings elsewhere (Ledger *et al*., 2008). *Ranunculus pseudofluitans* exhibited a reduced growth rate in both warmed and dewatered channels, whereas all three stressors combined increased the growth rate of *Berula erecta* (Chapter 3). This was hypothesised in Chapter 3 with the findings in agreement with Boulton (2003), suggesting that the direction of change in production is governed by the ability of taxa to tolerate amphibious conditions when streams and rivers dry.

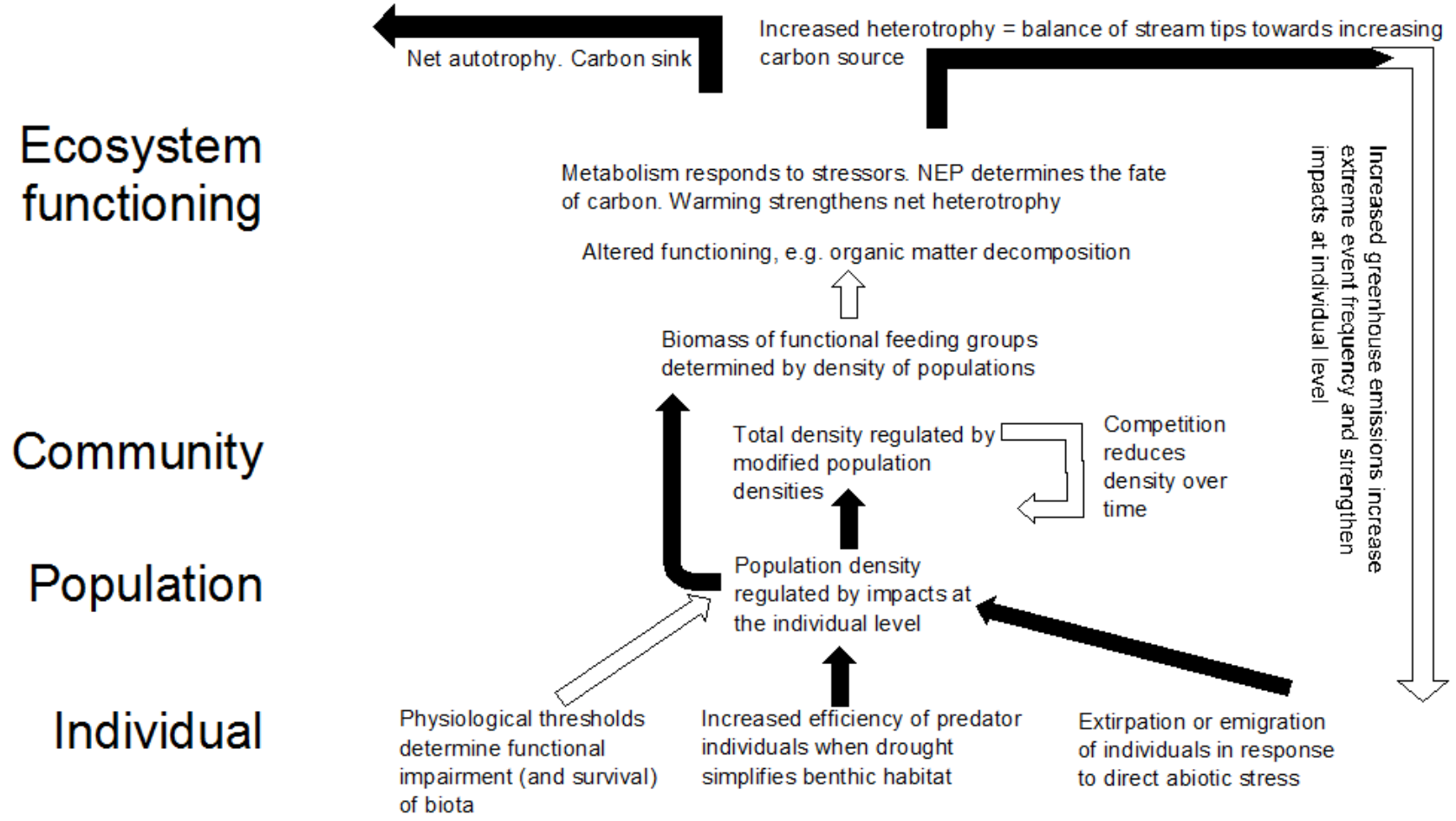
Stream metabolism is often governed by the responses of primary and secondary consumers within the system at an individual to community level (Allen *et al*., 2005). Determining the precise link between metabolism and lower ecological levels was beyond the remit of this research, but greater biomass of the grazer *Radix balthica* with warming may have driven down primary production and elevated secondary production, leading to an observed increase in net heterotrophy of warmed channels in Chapter 3. However this effect may equally have been due to elevated microbial densities which were not recorded within the boundaries of this research project. Elevated heterotrophy, as observed in warmed channels in Chapter 3, increases the role of stream and rivers as a net carbon source (Acuña *et al*., 2008; Boyero *et al*., 2011; Bruesewitz *et al*., 2013; O'Gorman *et al.*, 2012), and may consequently

lead to a positive feedback loop reinforcing effects through increased extreme event prevalence (IPCC, 2012). However, severe dewatering combined with warming and added sediment stress resulted in net autotrophy (Chapter 3), speculated to be attributable to conditions that exacerbate the release of limiting nutrients from sediment (House & Denison, 2000), enhancing primary production (Mainstone & Parr, 2002) and steering the stressed waterbody towards autotrophy.

By combining findings from the drought experiments in this research, it is possible to conceptualise the effect of drought at multiple levels of ecological complexity, and the links between them (Fig. 6.1). This emphasises the importance of understanding ecological effects at the simplest level in order to determine complex ecological responses.

It is hoped the research can be used to aid practitioners to set guidelines on river water temperatures, to prioritise stressors, to recognise the importance of river flow, and to further develop tools to develop a mechanistic understanding of ecological network impacts.

Figure 6.1. **Conceptualisation of drought stressor effects at multiple ecological levels.** Shaded arrows illustrate links evidenced within this research, non-shaded arrows illustrate inferred links. Diagram exemplifies the complexity of drought stress on stream and river ecology, and highlights how positive feedback loops may affect ecological responses to drought.



6.2. RIVER RESTORATION

River restoration is the process of improving degraded river channels, and returning lost channel elements, for a multitude of benefits including ecological processing (Wohl *et al*., 2015). Restoring and/or modifying river basins to adapt to climate change has proven challenging owing to the increased risk of hydrological extremes at both ends of the hydrological spectrum – i.e. floods and droughts (Cui *et al*., 2009). For example, channels can be straightened and dredged to cope with increased flow attributable to floods, but are then unable to retain water during periods of drought. Therefore, careful consideration should be given to maximise the best outcomes in a changing and variable future climate. Such strategies must be proactive (Palmer *et al*., 2009) rather than simply awaiting drought stressor impacts to materialise, in order to have the greatest chance of success, as hydrological extremes are unpredictable by nature and may give little warning – especially in the case of floods.

In the field of river restoration, much attention has been given to increasing suitable stream habitat; coined the ‘field of dreams’ hypothesis, whereby it is hoped if the habitat is there, ecological success will follow (Palmer *et al*., 1997). It would seem logical that for stream ecological processes to be maintained during drought, the greatest biodiversity should be achieved prior to the drought, and a plausible way of achieving this is through the provisioning of habitat heterogeneity, often lost in many rivers owing to straightening, dredging, removal of riparian vegetation etc. (Bond & Lake, 2003). It was evidenced in Chapters 2 and 3 that a reduction in habitat significantly reduced the density of a key taxon, *Gammarus pulex*, and the associated biomass of the shredder FFG, supporting the notion that a reduction in

suitable habitat is likely to reduce ecological success. Restoring watercourses and increasing habitat heterogeneity will also increase the likelihood of water retention in the channel following reduced flows and flow cessation, which the research in this thesis has demonstrated to be crucial for the survival of aquatic biota, strengthening the need to focus on habitat heterogeneity restoration. Channel naturalisation (e.g. un-straightening and connecting the channel to its floodplain) will undoubtedly help retain water in the channel and improve the river's ecological condition (Palmer *et al*., 2005).

Water reallocation has been shown to reduce the longitudinal distance of desiccated stream bed during periods of drought (Soulsby *et al*., 1999), whilst raising the level of small stream beds can reconnect the river laterally with its riparian zone during times of low flow (Querner & Van Lanen, 2001). Maintaining connectivity, both laterally and longitudinally is vital during drought to help maintain biotic community structure and functioning as movement of aquatic organisms principally occurs within the water column and along the wetted river bed (Bond & Lake, 2003; Weins, 1989). The most deleterious effects of drought (and drought compound events) can be avoided if sufficient water is retained in the channel. Reduced flow leads to a multitude of secondary stressors such as increased temperature variability, reduced DO, increased conductivity and modified pH (Bond *et al*., 2008; Boulton, 2003; Dollar *et al*., 2003; Lake, 2011), which would not otherwise occur if adequate flow can be maintained. Whilst this seems obvious, water managers must plan how to maintain sufficient flow during drought (e.g. sustainable abstractions, preservation of reservoir storage and augmentation schemes).

Sedimentation from erosion is expected to increase in future as the climate changes (Walling, 2009). The adverse in-channel effects of sedimentation evidenced throughout this research (e.g. reducing many taxa densities, elevating benthic respiration and reducing microbial decomposition) can be avoided by adopting a catchment wide approach to better manage land use and mitigate land-based sources of sediment entering the stream in the first instance. This would reduce the quantity of entrained sediment available for deposition during times of low flow. Alternatively, sediment traps may be used to stop sediment from entering streams and rivers (Environment Agency, 2010) whilst stabilising river banks may reduce sediment input from bank erosion (Envioronment Agency, 2011). Willow spiling can be used to stabilise banks, reducing sediment input into rivers prior to droughts, and increasing shading (Anstead *et al*., 2012). Such methods are sustainable and can last for 100 years, but are susceptible to cattle grazing and can rapidly die if drought occurs prior to the establishment of a suitable root stock (Anstead *et al*., 2012). Whether willow or a different riparian tree is used, it is crucial that the drought tolerance of the chosen riparian species is thoroughly investigated, owing to differences in susceptibility among species to reduced water availability (Singer *et al*., 2013). Moreover, provisions must be in place to ensure the success of newly implemented restoration measures, as unpredictable extremes may well occur prior to their establishment (Reich & Lake, 2015). Where no easy solution can prevent sediment input to the river, knowledge of compound sediment effects when combined with additional stressors should be utilised to target management strategies more effectively. For example where sediment combined with a second stressor produces synergistic deleterious effects, it may be more feasible to attempt

to mitigate or prevent the second stressor in an attempt to reduce the overall impact caused by sediment. Likewise, where deleterious effects arise for other co-occurring stressor combinations, it may be possible to restore ecological health by tackling the easiest stressor. For example, where dewatering and warming together reduced collector biomass in Chapter 3, this could be prevented by channel shading alone, if the reallocation of water to the channel to increase habitat area, is not feasible.

It is possible to make predictions on the outcome of drought, and to make management decisions, based on knowledge of the requirements and ecological niches of individual taxa (Crook *et al*., 2010). For example, the sensitivity of bullhead to water temperature and physico-chemical deterioration, along with its predatory impact and predatory susceptibility can determine both the requirements needed during drought to support this taxon, as well as the altered risk posed to the macroinvertebrate community. Where the ecology of susceptible taxa in drought-risk localities is poorly understood, improved efforts should be made to better understanding, so that biotic information can be fed into management plans to ensure ecological achievement.

Pools can provide critical refugia during drought (e.g. Labbe & Fausch, 2000). It should be ensured that these are therefore provided prior to drought occurrence, which may be carried out directly by deepening, indirectly by allowing flow heterogeneity, caused by large woody debris, to naturally produce pools (Larson *et al*., 2001) or by reducing abstractions in an attempt to increase water depth in pools. However, water temperature – perhaps the most problematic stressor which has been shown to frequently interact with other stressors in Chapter 2, may lead to mortality of taxa seeking refuge in pools (Tramer, 1977; Verdonschot *et al*., 2015).

As sensible heat will increase water temperatures via equilibrium with the surrounding air (Hannah & Garner, 2015; Webb & Zhang, 2004), there is no easy fix to prevent remnant water body temperature from rising. However there is unequivocal evidence that overhanging riparian vegetation can, through provisioning of shade, lower water temperatures and prevent critical ecological thermal thresholds from being breached (Broadmeadow *et al*., 2011; Davies, 2010; Mantyka-Pringle *et al*., 2014). Although the effects of shade on water temperature were not investigated in Chapter 5, the isolated pool investigated was subjected to direct insolation, and it is believed that shading would have lowered water temperature in this pool below the CTmax of >50% of taxa investigated. Fencing can also be implemented around pools and along riparian corridors to prevent deleterious cattle effects on terrestrial vegetation which in turn provides shade during times of drought, hot days, and heat waves (Davies, 2010) and reduces poaching effects. As water volume affects its thermal capacity (Hannah & Garner, 2015) all efforts should be made to maximise pool water depth. One possible method of doing this may be periodic flow augmentation to refill shrinking pools, where resource availability allows.

Priority should be given to larger refugia units where possible, as larger refugia are typically more resistant to disturbance (Sedell *et al*., 1990). The scale of implementation is equally critical to the success of the restoration, with riparian shading of ~300m needed to reduce water temperatures in a study of New Zealand streams (Storey & Cowley, 1997). Practitioners should therefore be mindful of the scale of restoration measures to ensure that they will achieve the desired outcome. Whilst small pools (i.e. outdoor mesocosms) in Chapter 2 were sufficient for a large

proportion of the initial macroinvertebrate community to persist, the speed of re-colonisation along the length of the channel following drought would rely upon both the number and connectedness of such refugia. Moreover it should be remembered that the stream and its catchment are connected (Hynes, 1975), and thus uncoupling of the stream may result in the failure of in-stream restoration techniques. For examples, trees in the catchment aid the percolation of water which in turn elevates base flow during periods of reduced rainfall (Thomson *et al*., 2012). As such, the planting of trees in the catchment and the removal of impermeable surfaces will greatly increase the success of all in-stream restoration attempts.

Education of landowners pertaining to restoration and their subsequent involvement will be of great benefit to river restoration and river ecosystem health during droughts. For example, during drought, landowners could reduce water abstraction volumes, and ensure the presence of deep pools within the rivers, to enable connection of refugia to up and downstream sections. This thesis illustrates that pools provide refugia for biota during drought (remnant communities persisted for six weeks in outdoor near-lentic mesocosms), highlighting the importance of pools in preventing extirpation when the river dries. Thus, ensuring deep pools are prevalent along the course of the river prior to droughts will be advantageous to benthic ecology following flow cessation (Reich & Lake, 2015). However despite every best effort to mitigate effects, hydrologic drought may still continue to be an inevitable phenomenon that will have adverse ecological effects on the ecology of running waters. That said, the increased incidence of droughts over a longer temporal period may lead to evolutionary adaptations of taxa to withstand or avoid the heightened stress (Bonada *et al*., 2007; Douglas *et al*., 2003).

It is hoped that the research in this thesis can be utilised to help bridge gaps between science and management, and to overcome existing challenges in understanding how restoration efforts may improve stream ecology at multiple hierarchical levels including productivity and metabolism (Wohl *et al*., 2015).

6.3 SUGGESTIONS FOR FURTHER RESEARCH

Future work is suggested based on findings from this research project and continued research gaps.

- **Physiological thresholds as a tool to predict extreme event impacts on aquatic food webs**. Species loss to disturbance is non-random (Jonsson *et al*., 2002), but instead dictated by sensitivity of different species to stress. In the case of temperature, species loss will obviously be determined by sensitivity to extreme maxima and minima (Dallas & Ketley, 2011; Dallas & Rivers-Moore, 2012). The development of a whole stream system taxa thermal physiology database is an important deterministic tool to predict differential vulnerability of taxa to warming (e.g. CTmax, extirpation) and may have applications in the assessment of food web robustness. Future studies should derive physiological thresholds across entire stream assemblages, as these data are much more meaningful when incorporating a greater proportion of the community.
- **Quantifying predator impacts under multiple drought stressors**. The feeding experiment used in this research proved a useful mechanistic tool to determine altered biotic interactions in response to habitat modification. But

many questions now come to light: How would the intensified predator pressure of sediment addition be affected by the addition of a second stressor, e.g. warming? Do predator-prey interactions weaken during drought when water quality deteriorates? Would prey-switching prevent extinction of a single prey population, if additional prey populations were available for consumption alongside? The feeding experiment conducted as part of this research has provided evidence for intensified predation pressure that was previously only speculation. Further research should adopt the use of this same technique as a useful tool to predict global change (O'Gorman, 2014), and should test these newly emerged questions to better understanding further.

- **Linearity of drought stressors.** This research project has paved the way in determining independent and interactive effects of drought stressors on an array of ecological receptors. But at what point does sediment elicit adverse effects, and are effects more beneficial at reduced sediment quantities? Dewatering effects were relatively weak in the multiple stressor experiment (Chapters 2 and 3), but flow cessation and stream-bed desiccation have been reported to invoke severe effects on richness elsewhere (Boersma *et al*., 2014; Boulton, 2003; Calapez *et al*., 2014). Do the applied stressors produce non-linear effects along applied stressor gradients? Further research should investigate non-linear impacts of drought stressors not yet tested (e.g. aquatic habitat loss) when applied singly and in combination to build on the current findings from this research and assist implementation of critical thresholds for community structure and functional processes.

6.4 CONCLUSION

This research has identified drought effects across multiple levels of ecological complexity, and has gone some way to better understanding of drought impact causal mechanisms. The research has been conducted using small scale experiments allowing carefully controlled manipulations of abiotic parameters. The challenge now is to extrapolate these findings to natural systems and to implement the findings into policy guidelines. Moreover, research relating individual effects and ecosystem processes is in its infancy and requires immediate attention. Further research should use both larger spatial and temporal experiments and take advantage of naturally occurring hydrologic drought in order to depict a greater overall picture of extreme event impacts on community structure and ecosystem functioning at multiple levels of ecological complexity.

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APPENDICES

APPENDIX A

Supplementary material to accompany Chapter Two.

Table A1. **Comprehensive macroinvertebrate taxa list from the mesocosm experiment.** Taxa identified from Surber samples collected on day 42 to lowest practicable taxonomic unit.

----- Taxon -----	
Agapetus fuscipes	*Limnius volckmari* (larvae)
Ancylus fluviatilis	Metriocnemus eurynotus type
Asellidae sp.	*Nemurella picteti*
Asellus aquaticus	Oligochaeta spp.
Baetis rhodani	*Corynoneura* sp.
Baetis sp.	Orthocladinae sp.
Baetis vernus	Orthocladius S type/ Paratrichcladius
Caenis luctuosa	*Pisidium* sp.
Caenis pusilla	*Planaria torva*
Caenis rivulorum	*Planorbis planorbis*
Caenis sp.	*Polycelis felina*
Ceratopogonidae sp.	*Polycelis nigra/Polycelius tenuis*
Chaetocladius Dentiforceps type	*Prodiamesa* sp.
Drusus anulatus	*Radix balthica*
Dugesia lugubris/Dugesia polychroa	*Sericostoma personatum*
Dytiscidae sp. (larvae)	*Serratella ignita*
Elmis aenea (adult)	*Sialis lutaria*
Elmis aenea (larvae)	*Silo nigricornis*
Empididae sp.	*Silo* sp. (Instar II)
Ephemera danica	*Synorthocladius semivirens*
Erpobdella octoculata	*Ablabesmyia* sp.
Gammarus pulex	*Macropelopia* sp.
Helobdella stagnalis	*Microspectra* type
Helophorus sp.	*Tinodes waeneri*
Lepidostoma hirtum	*Tipula* sp.
Leuctra geniculata	*Valvata macrostomata*
Limnephilidae sp.	
Limnius volckmari (adult)	

Tables A2. **Three way ANOVA output tables for statistical tests conducted on community level (taxon richness, total abundance) and population level (taxon density) variables.** Far left column illustrates the main effect (first three rows) and interaction effect (subsequent four rows) tested for each model.

Caenis luctuosa					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.093	0.0930	0.593	0.44726
Sedimentation	1	0.091	0.0909	0.579	0.45250
Dewatering	1	0.606	0.6063	3.862	0.05841
Temperature : Sedimentation	1	0.322	0.3220	2.051	0.16212
Temperature : Dewatering	1	2.065	2.0650	13.151	0.00102
Sedimentation : Dewatering	1	0.133	0.1331	0.848	0.36436
Temperature : Sedimentation : Dewatering		0.955	0.9549	6.082	0.01939

Chaetocladius dentiforceps type					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	2.488	2.4884	3.501	0.0718
Sedimentation	1	0.193	0.1925	0.271	0.6068
Dewatering	1	0.032	0.0321	0.045	0.8332
Temperature : Sedimentation	1	0.025	0.0248	0.035	0.8532
Temperature : Dewatering	1	0.312	0.3119	0.439	0.5131
Sedimentation : Dewatering	1	0.363	0.3628	0.510	0.4808
Temperature : Sedimentation : Dewatering		0.178	0.1782	0.251	0.6205

Gammarus pulex					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.954	0.9536	5.843	0.02215
Sedimentation	1	0.376	0.3759	2.303	0.13994
Dewatering	1	1.296	1.2958	7.940	0.00862
Temperature : Sedimentation	1	0.728	0.7275	4.458	0.04347
Temperature : Dewatering	1	0.006	0.0062	0.038	0.84686
Sedimentation : Dewatering	1	0.162	0.1623	0.995	0.32685
Temperature : Sedimentation : Dewatering		0.381	0.3811	2.336	0.13729

Tables A2 continued. **Three way ANOVA output tables for statistical tests conducted on community level (taxon richness, total abundance) and population level (taxon density) variables.** Far left column illustrates the main effect (first three rows) and interaction effect (subsequent four rows) tested for each model.

Helobdella stagnalis					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	1.456	1.4565	3.347	0.0776
Sedimentation	1	0.353	0.3528	0.811	0.3753
Dewatering	1	1.183	1.1826	2.717	0.1101
Temperature : Sedimentation	1	0.061	0.0606	0.139	0.7119
Temperature : Dewatering	1	0.111	0.1110	0.255	0.6173
Sedimentation : Dewatering	1	0.838	0.8383	1.926	0.1757
Temperature : Sedimentation : Dewatering		0.051	0.0510	0.117	0.7346

Macropelopia sp.					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.271	0.2710	0.849	0.3644
Sedimentation	1	0.707	0.7072	2.215	0.1474
Dewatering	1	0.108	0.1076	0.337	0.5660
Temperature : Sedimentation	1	0.051	0.0505	0.158	0.6937
Temperature : Dewatering	1	0.001	0.0009	0.003	0.9585
Sedimentation : Dewatering	1	1.131	1.1308	3.542	0.0699
Temperature : Sedimentation : Dewatering		1.266	1.2657	3.965	0.0560

Micropsectra sp.					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.0547	0.0547	0.623	0.43590
Sedimentation	1	2.5238	2.5238	28.716	7e-06
Dewatering	1	0.0000	0.0000	0.000	0.99993
Temperature : Sedimentation	1	0.0022	0.0022	0.025	0.87595
Temperature : Dewatering	1	0.8019	0.8019	9.124	0.00493
Sedimentation : Dewatering	1	0.0041	0.0041	0.047	0.82978
Temperature : Sedimentation : Dewatering		0.0227	0.0227	0.258	0.61497

Tables A2 continued. **Three way ANOVA output tables for statistical tests conducted on community level (taxon richness, total abundance) and population level (taxon density) variables.** Far left column illustrates the main effect (first three rows) and interaction effect (subsequent four rows) tested for each model.

Oligochaeta spp.					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.226	0.2259	1.175	0.288
Sedimentation	1	0.207	0.2073	1.075	0.309
Dewatering	1	0.471	0.4709	2.443	0.129
Temperature : Sedimentation	1	0.414	0.4141	2.149	0.154
Temperature : Dewatering	1	0.157	0.1571	0.815	0.374
Sedimentation : Dewatering	1	0.011	0.0113	0.059	0.810
Temperature : Sedimentation : Dewatering		0.271	0.2712	1.407	0.245

Orthocladius S-type					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.017	0.0165	0.024	0.877
Sedimentation	1	0.896	0.8961	1.313	0.261
Dewatering	1	0.472	0.4724	0.692	0.412
Temperature : Sedimentation	1	0.010	0.0102	0.015	0.904
Temperature : Dewatering	1	0.371	0.3709	0.543	0.467
Sedimentation : Dewatering	1	0.047	0.0475	0.070	0.794
Temperature : Sedimentation : Dewatering		1.425	1.4255	2.088	0.159

Polycelis nigra					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.091	0.091	0.248	0.622
Sedimentation	1	8.556	8.556	23.220	4.55e-05
Dewatering	1	0.368	0.368	0.998	0.326
Temperature : Sedimentation	1	0.070	0.070	0.189	0.667
Temperature : Dewatering	1	0.832	0.832	2.257	0.144
Sedimentation : Dewatering	1	0.000	0.000	0.000	0.983
Temperature : Sedimentation : Dewatering		0.209	0.209	0.567	0.458

Tables A2 continued. **Three way ANOVA output tables for statistical tests conducted on community level (taxon richness, total abundance) and population level (taxon density) variables.** Far left column illustrates the main effect (first three rows) and interaction effect (subsequent four rows) tested for each model.

Radix balthica

	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.8486	0.8486	18.461	0.000178
Sedimentation	1	0.0726	0.0726	1.579	0.218966
Dewatering	1	0.0438	0.0438	0.952	0.337283
Temperature : Sedimentation	1	0.2453	0.2453	5.336	0.028204
Temperature : Dewatering	1	0.0040	0.0040	0.087	0.770393
Sedimentation : Dewatering	1	0.0019	0.0019	0.041	0.841648
Temperature : Sedimentation : Dewatering		0.0020	0.0020	0.044	0.835334

Serratella ignita

	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.111	0.1111	0.266	0.610
Sedimentation	1	0.930	0.9298	2.228	.0147
Dewatering	1	1.086	1.0862	2.603	0.118
Temperature : Sedimentation	1	0.123	0.1228	0.294	0.592
Temperature : Dewatering	1	1.166	1.1661	2.794	0.106
Sedimentation : Dewatering	1	0.029	0.0285	0.068	0.796
Temperature : Sedimentation : Dewatering		0.028	0.0280	0.067	0.798

Synorthocladius semivirens

	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.076	0.076	0.192	0.664151
Sedimentation	1	1.217	1.217	3.079	0.089875
Dewatering	1	0.794	0.794	2.008	0.167112
Temperature : Sedimentation	1	6.735	6.735	17.032	0.000283
Temperature : Dewatering	1	0.001	0.001	0.001	0.971850
Sedimentation : Dewatering	1	0.050	0.050	0.127	0.724571
Temperature : Sedimentation : Dewatering		1.659	1.659	4.195	0.049695

Tables A2 continued. **Three way ANOVA output tables for statistical tests conducted on community level (taxon richness, total abundance) and population level (taxon density) variables.** Far left column illustrates the main effect (first three rows) and interaction effect (subsequent four rows) tested for each model.

SPECIES RICHNESS					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.23	0.23	0.024	0.8779
Sedimentation	1	34.22	34.22	3.646	0.0652
Dewatering	1	3.03	3.03	0.322	0.5742
Temperature : Sedimentation	1	21.03	21.03	2.240	0.1443
Temperature : Dewatering	1	13.22	13.22	1.409	0.2440
Sedimentation : Dewatering	1	4.22	4.22	0.450	0.5071
Temperature : Sedimentation : Dewatering		0.03	0.03	0.003	0.9592

TOTAL ABUNDANCE					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.1158	0.1158	4.823	0.036235
Sedimentation	1	0.0447	0.0447	1.863	0.182736
Dewatering	1	0.0000	0.0000	0.000	0.987897
Temperature : Sedimentation	1	0.2624	0.2624	10.926	0.00251
Temperature : Dewatering	1	0.3295	0.3295	13.722	0.000887
Sedimentation : Dewatering	1	0.0160	0.0160	0.667	0.420764
Temperature : Sedimentation : Dewatering		0.0089	0.0089	0.370	0.547829

Table A3. **Comprehensive list of taxa recorded in the surrounding locality.** Merged taxa list of samples taken from the River Itchen, Candover Brook and farm feeder channels. Taxa in bold were not recorded in the mesocosms following the 42 day long experiment. Ordered alphabetically by major group.

Major Group	Taxon
Annelida (Hirudinea)	***Glossiphonia heteroclita***
Annelida (Hirudinea)	***Erpobdella octoculata***
Annelida (Hirudinea)	*Helobdella stagnalis*
Annelida (Hirudinea)	***Piscicola geometra***
Annelida (Oligochaeta)	Oligochaeta spp.
Coleoptera	*Elmis aenea*
Coleoptera	*Limnius volckmari*
Coleoptera	***Orectochilus villosus***
Coleoptera	Dytiscidae sp.
Coleoptera	***Oreodytes sanmarkii***
Crustacea	*Gammarus pulex*
Crustacea	*Asellus aquaticus*
Diptera	**Simuliidae sp.**
Diptera	Chironomidae spp.
Diptera	**Ephydridae sp.**
Diptera	**Pediciidae sp.**
Diptera	**Physidae sp.**
Diptera	*Ceratopogonidae* sp.
Diptera	Tipulidae sp.
Ephemeroptera	***Heptagenia sulphurea***
Ephemeroptera	*Serratella ignita*
Ephemeroptera	***Baetis buceratus***
Ephemeroptera	*Ephemera danica*
Ephemeroptera	*Baetis rhodani*
Ephemeroptera	*Caenis pusilla*
Ephemeroptera	***Electrogena lateralis***

Table A3 continued. **Comprehensive list of taxa recorded in the surrounding locality.** Merged taxa list of samples taken from the River Itchen, Candover Brook and farm feeder channels. Taxa in bold were not recorded in the mesocosms following the 42 day long experiment. Ordered alphabetically by major group.

Major Group	Taxon
Megaloptera	***Sialis lutaria***
Mollusca	*Ancylus fluviatilis*
Mollusca	*Radix balthica*
Mollusca	*Planorbis planorbis*
Odonata (Zygoptera)	***Calopteryx virgo***
Plecoptera	***Leuctra nigra***
Plecoptera	***Nemoura cambria / erratica***
Plecoptera	*Nemurella picteti*
Trichoptera	***Hydropsyche pellucidula***
Trichoptera	*Drusus annulatus*
Trichoptera	***Hydropsyche siltalai***
Trichoptera	*Silo nigricornis*
Trichoptera	*Agapetus fuscipes*
Trichoptera	***Odontocerum albicorne***
Trichoptera	***Sericostoma personatum***
Trichoptera	***Rhyachophila dorsalis***
Trichoptera	***Potamophylax rotundipennis***
Trichoptera	***Polycentropus flavomaculatus***
Trichoptera	***Rhyacophila septentrionis***
Trichoptera	**Goeridae sp.**
Triclada	*Polycelis nigra/tenuis*
Triclada	*Polycelis felina*
Triclada	*Planaria torva*
Triclada	*Dugesia lugubris / polychroa*

APPENDIX B

Supplementary material to accompany Chapter Three.

Table B1. **Sources of length-mass equations for the determination of macroinvertebrate biomass estimation.** Right side column shows taxa for which the referenced source contained equations.

Source	Taxa covered
Benke, A. C., Huryn, A. D., Smock, L. A. & Wallace, J. B. (1999). Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *Journal of the North American Benthological Society*, **18**, 308-343.	*Pisidium* spp.; *Caenis luctuosa; Caenis pusilla; Caenis rivulorum; Tipulidae* spp.*; Planaria torva; Lepidostoma hirtum*
Burgherr, P. & Meyer, E. I. (1997). Regression analysis of linear body dimensions vs. dry mass in stream macroinvertebrates. *Archiv für Hydrobiologie*, **139**, 101-112.	*Ephemera danica; Baetis rhodani; Baetis vernus; Baetis* spp.; *Gammarus pulex; Leuctra geniculate; Nemurella picteti; Limnius volckmari* (adult and larvae); *Elmis aenea* (adult and larvae)
Edwards, F. K., Lauridsen, R. B., Armand, L., Vincent, H. M. & Jones, J. I. (2009). The relationship between length, mass and preservation time for three species of freshwater leeches (Hirudinea). *Fundamental and Applied Limnology*, **173**, 321-327.	*Erpobdella octoculata; Helobdella stagnalis;*
Johnston, T. A. & Cunjak, R. A. (1999). Dry mass-length relationships for benthic insects: a review with new data from Catamaran Brook, New Brunswick, Canada. *Freshwater Biology*, **41**, 653-674.	*Sialis lutaria;*
Mason, C. F. (1977). Populations and production of benthic animals in two contrasting shallow lakes in Norfolk. *Journal of Animal Ecology*, **46**, 147-172.	*Planorbis planorbis; Valvata macrostomata; Oligochaeta* spp.; *Asellus aquaticus; Asellidae* spp.*;*

Table B1 continued. **Sources of length-mass equations for the determination of macroinvertebrate biomass estimation.** Right side column shows taxa for which the referenced source contained equations.

Meyer, E. (1989). The relationship between body length parameters and dry mass in running water invertebrates. *Archiv für Hydrobiologie*, **117**, 191-203.	*Ancylus fluviatilis; Serratella ignita; Ceratopogonidae* spp.*; Empididae* spp.; *Polycelis felina; Polycelis nigra / tenuis; Polycelis* spp.*; Limnephilidae* spp.*; Drusus annulatus; Tinodes waeneri; Agapetus fuscipes; Sericostoma personatum; Silo nigricornis; Silo* spp.; *Dytiscidae* spp. (larvae); *Helophoridae* spp. (larvae); Chironominae (Tanytarsini); Tanypodinae; Prodiamesinae; Orthocladiinae;
Vincent, Helen. Unpublished.	*Radix balthica;*

Tables B2. **Three way ANOVA output tables for statistical tests conducted on functional feeding groups.** Far left column illustrates the main effect (first three rows) and interaction effect (subsequent four rows) tested for each model.

Total biomass					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.2198	0.2198	2.894	0.0996
Sedimentation	1	0.0020	0.0020	0.027	0.8716
Dewatering	1	0.6454	0.0645	0.849	0.3644
Temperature : Sedimentation	1	0.4651	0.4651	6.124	0.0194
Temperature : Dewatering	1	0.0445	0.0445	0.586	0.4500
Sedimentation : Dewatering	1	0.0196	0.0196	0.259	0.6149
Temperature : Sedimentation : Dewatering		0.0655	0.0655	0.862	0.3607

Collectors					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.0207	0.0207	0.543	0.466938
Sedimentation	1	0.0413	0.0413	1.085	0.306257
Dewatering	1	0.0118	0.0118	0.309	0.582429
Temperature : Sedimentation	1	0.0988	0.0988	2.592	0.118245
Temperature : Dewatering	1	0.7393	0.7393	19.402	0.000132
Sedimentation : Dewatering	1	0.0281	0.0281	0.737	0.397614
Temperature : Sedimentation : Dewatering		0.0611	0.0611	1.604	0.215352

Grazers					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	1.982	1.9823	5.979	0.0208
Sedimentation	1	0.1280	0.1280	0.386	0.5392
Dewatering	1	0.000	0.0002	0.000	0.9826
Temperature : Sedimentation	1	0.515	0.5147	1.553	0.2227
Temperature : Dewatering	1	0.464	0.4641	1.400	0.2464
Sedimentation : Dewatering	1	0.054	0.0538	0.162	0.6900
Temperature : Sedimentation : Dewatering		0.376	0.3759	1.134	0.2957

Tables B2. **Three way ANOVA output tables for statistical tests conducted on functional feeding groups.** Far left column illustrates the main effect (first three rows) and interaction effect (subsequent four rows) tested for each model.

Predators					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.899	0.8900	2.842	0.103
Sedimentation	1	0.092	0.0919	0.290	0.594
Dewatering	1	0.446	0.4464	1.1411	0.245
Temperature : Sedimentation	1	0.372	0.3717	1.175	0.287
Temperature : Dewatering	1	0.433	0.4326	1.368	0.252
Sedimentation : Dewatering	1	0.030	0.0301	0.095	0.760
Temperature : Sedimentation : Dewatering		0.079	0.0789	0.250	0.621

Shredders					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.288	0.288	0.440	0.5123
Sedimentation	1	2.546	2.546	3.886	0.0583
Dewatering	1	3.168	3.168	4.835	0.0360
Temperature : Sedimentation	1	1.626	1.626	2.482	0.1260
Temperature : Dewatering	1	1.186	1.186	1.809	0.1890
Sedimentation : Dewatering	1	0.121	0.121	0.185	0.6705
Temperature : Sedimentation : Dewatering		0.689	0.689	1.051	0.3138

Table B3. **Three way ANOVA output tables for statistical tests conducted on macrophyte parameters.** Far left column illustrates the main effect (first three rows) and interaction effect (subsequent four rows) tested for each model.

Ranunculus RRG					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.00016	0.00016	6.213	0.0180
Sedimentation	1	0.00001	0.00003	1.363	0.2517
Dewatering	1	0.00052	0.00052	20.658	7.41e-05
Temperature : Sedimentation	1	0.00000	0.00000	0.060	0.8076
Temperature : Dewatering	1	0.00000	0.00000	0.071	0.7918
Sedimentation : Dewatering	1	0.00012	0.00012	4.839	0.0352
Temperature : Sedimentation : Dewatering		0.00000	0.00000	0.068	0.7967

Berula RRG					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	3.704e-1	3.704e-1	5.079	0.0317
Sedimentation	1	4.800e-1	4.800e-1	0.066	0.7993
Dewatering	1	2.600e-1	2.600e-1	0.357	0.5547
Temperature : Sedimentation	1	4.120e-1	4.120e-1	0.564	0.4584
Temperature : Dewatering	1	1.408e-1	1.408e-1	1.930	0.1750
Sedimentation : Dewatering	1	1.052e-1	1.052e-1	1.442	0.2392
Temperature : Sedimentation : Dewatering		5.635e-1	5.635e-1	7.726	0.0093

Table B3. **Three way ANOVA output tables for statistical tests conducted on macrophyte parameters.** Far left column illustrates the main effect (first three rows) and interaction effect (subsequent four rows) tested for each model.

Ranunculus chlorophyll a					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.0001	0.0001	0.006	0.93751
Sedimentation	1	0.0000	0.0001	0.003	0.95702
Dewatering	1	0.0907	0.0907	7.722	0.00905
Temperature : Sedimentation	1	0.0000	0.0001	0.001	0.98114
Temperature : Dewatering	1	0.0020	0.0020	0.174	0.67931
Sedimentation : Dewatering	1	0.0161	0.0161	1.371	0.25034
Temperature : Sedimentation : Dewatering		0.0029	0.0029	0.245	0.62391

Berula chlorophyll a					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.01499	0.014992	5.355	0.0272
Sedimentation	1	0.01196	0.011962	4.273	0.0469
Dewatering	1	0.00001	0.000001	0.003	0.9561
Temperature : Sedimentation	1	0.00012	0.000124	0.044	0.8348
Temperature : Dewatering	1	0.00042	0.00421	0.150	0.7008
Sedimentation : Dewatering	1	0.00930	0.004321	1.544	0.2231
Temperature : Sedimentation : Dewatering		0.00930	0.009300	3.322	0.0777

Figure B1. **Interaction plots illustrating the three way interaction affecting *B. erecta* RGR.** Data points represent treatment mean. Coloured bars join together data points of the same temperature level (orange = warmed, blue = ambient). Codes represent treatments, where C= control, W = warmed, S = silt and D = dewatered. Treatments with a mean RGR > control are in bold. The two plots together explain how the effect of sediment on warming (to decrease W mean) is dependent upon the level of dewatering (where dewatering eliminates the negative effect sediment has on warming).

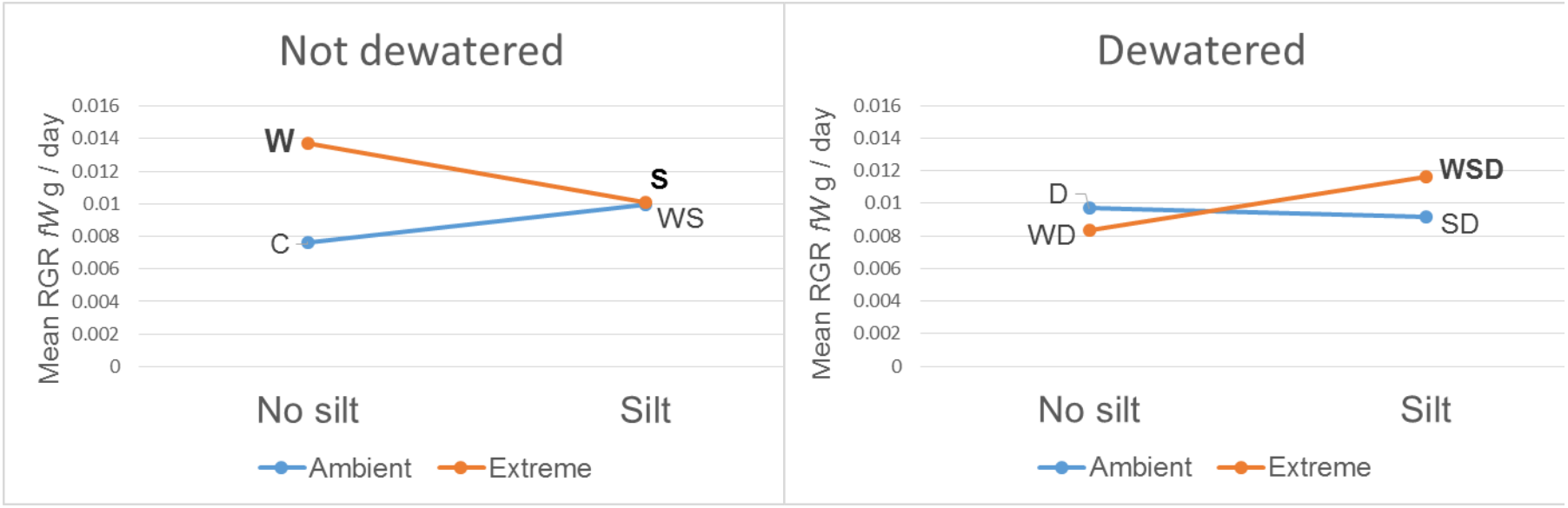


Table B4. **Three way ANOVA output tables for statistical tests conducted on metabolism parameters.** Far left column illustrates the main effect (first three rows) and interaction effect (subsequent four rows) tested for each model.

Benthic respiration experiment					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.164	0.1640	1.554	0.22191
Sedimentation	1	1.626	1.6264	15.406	0.00045
Dewatering	1	0.054	0.0536	0.508	0.48148
Temperature : Sedimentation	1	0.000	0.0003	.003	0.95833
Temperature : Dewatering	1	0.355	0.3546	3.359	0.07645
Sedimentation : Dewatering	1	0.134	0.1346	1.265	0.26933
Temperature : Sedimentation : Dewatering		0.065	0.0653	0.619	0.43741

GPP					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.306	0.3055	1.397	0.2519
Sedimentation	1	0.044	0.0442	0.202	0.6583
Dewatering	1	0.976	0.9757	4.460	0.0482
Temperature : Sedimentation	1	0.103	0.1029	0.470	0.5011
Temperature : Dewatering	1	0.267	0.2674	1.222	0.2827
Sedimentation : Dewatering	1	0.001	0.0008	0.003	0.9536
Temperature : Sedimentation : Dewatering		0.183	0.1832	0.837	0.3716

ER					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.3801	0.3801	2.648	0.120
Sedimentation	1	0.0068	0.0068	0.047	0.830
Dewatering	1	0.3652	0.3652	2.544	0.127
Temperature : Sedimentation	1	0.0215	0.0215	0.150	0.703
Temperature : Dewatering	1	0.0156	0.0156	0.109	0.745
Sedimentation : Dewatering	1	0.0052	0.0052	0.03	0.850
Temperature : Sedimentation : Dewatering		0.0722	0.0722	0.503	0.487

Table B4 continued. **Three way ANOVA output tables for statistical tests conducted on metabolism parameters.** Far left column illustrates the main effect (first three rows) and interaction effect (subsequent four rows) tested for each model.

ER:GPP					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.0041	0.00407	0.071	0.793
Sedimentation	1	0.0163	0.01632	0.284	0.600
Dewatering	1	0.1470	0.14704	2.561	0.126
Temperature : Sedimentation	1	0.0303	0.03035	0.528	0.476
Temperature : Dewatering	1	0.1539	0.015388	2.680	0.118
Sedimentation : Dewatering	1	0.0100	0.01000	0.174	0.681
Temperature : Sedimentation : Dewatering		0.0254	0.02538	0.442	0.514

Table B5. **Three way ANOVA output tables for statistical tests conducted on decomposition parameters.** Far left column illustrates the main effect (first three rows) and interaction effect (subsequent four rows) tested for each model.

Ktotal					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.86405	0.00001	0.030	0.86405
Sedimentation	1	0.00144	0.00250	12.398	0.00144
Dewatering	1	0.59274	0.00006	0.293	0.59274
Temperature : Sedimentation	1	0.47180	0.00011	0.532	0.47180
Temperature : Dewatering	1	0.36931	0.00002	0.832	0.36931
Sedimentation : Dewatering	1	0.71309	0.00003	0.138	0.71309
Temperature : Sedimentation : Dewatering		0.68772	0.00003	0.165	0.68772

Kinvert					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.00007	0.00007	0.207	0.653
Sedimentation	1	0.00059	0.00059	1.878	0.181
Dewatering	1	0.00001	0.00001	0.010	0.920
Temperature : Sedimentation	1	0.00001	0.00001	0.020	0.888
Temperature : Dewatering	1	0.00001	0.00001	0.010	0.920
Sedimentation : Dewatering	1	0.00001	0.00001	0.005	0.943
Temperature : Sedimentation : Dewatering		0.00022	0.00022	0.703	0.409

Kmicrobe					
	Df	**Sum sq.**	**Mean Sq.**	**F value**	***P***
Temperature	1	0.00001	0.000001	0.003	0.959
Sedimentation	1	0.00134	0.001338	60.375	1.14e-08
Dewatering	1	0.00001	0.000035	1.591	0.217
Temperature : Sedimentation	1	0.00001	0.000001	0.019	0.892
Temperature : Dewatering	1	0.00004	0.000043	1.960	0.172
Sedimentation : Dewatering	1	0.00003	0.000026	1.162	0.290
Temperature : Sedimentation : Dewatering		0.00001	0.000001	0.081	0.778

APPENDIX C

Supplementary material to accompany Chapter Four.

Table C1a. **Proportional distribution of substrata used in the control feeding experiment mesocosm.** Proportions obtained following dry sieving with a vibratory sieve shaker (Fritsch, Idar-Oberstein, Germany).

Control Treatment

phi scale	**size categories**	**sieve**	**% tot**
	>64.1		25.84189855
pebbles	4.1-64	4	70.22854517
granules	2.1-4	2	3.593214674
sand very coarse	1.1-2	1	0.173393499
sand coarse	0.51-1	0.5	0.033425253
sand medium	0.251-0.5	0.25	0.087741289
sand fine	0.1251-0.25	0.125	0.041781566
sand very fine	0.0631-0.125	0.063	

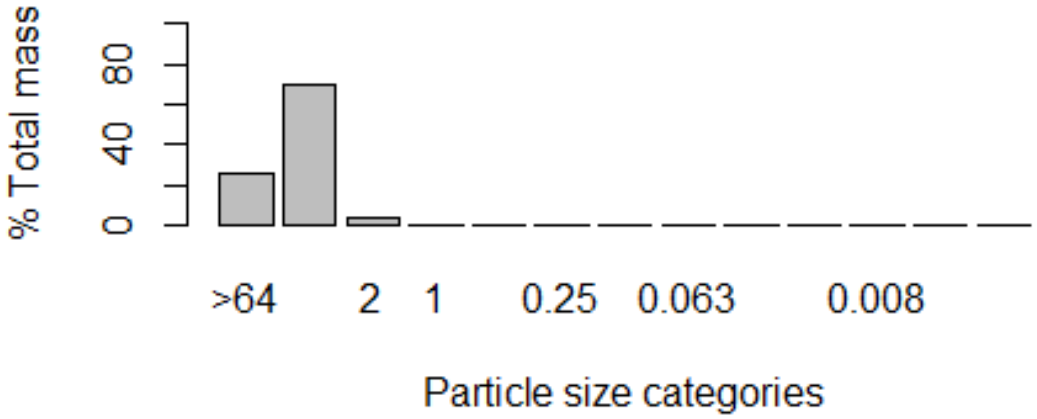
Table C1b. **Proportional distribution of substrata used in the control feeding experiment mesocosm.** Proportions obtained from dry sieving using a Fritsch vibratory sieve shaker (Fritsch, Idar-Oberstein, Germany).

Sediment Treatment

phi scale	**size categories**	**sieve**	**% tot**
	>2mm	2	0.044948309
sand very coarse	1.1-2	1	0.104879389
sand coarse	0.51-1	0.5	4.000399541
sand medium	0.251-0.5	0.25	71.08325426
sand fine	0.1251-0.25	0.125	24.67662188
sand very fine	0.0631-0.125	0.063	0.084902362
silt	0.0321-0.063	0.032	0.004994257
silt	0.0161-0.032	0.016	0
silt	0.0081-0.016	0.008	0
silt	0.0041-0.008	0.004	0
clay	<0.004	base pan	0

Figure C1. **Frequency distribution of substrata used in the feeding experiment mesocosms.** Showing a) Control and b) sediment treatments. Sediment mass calculated following dry sieving and sorting into sieves of varying mesh size.

a)



b)

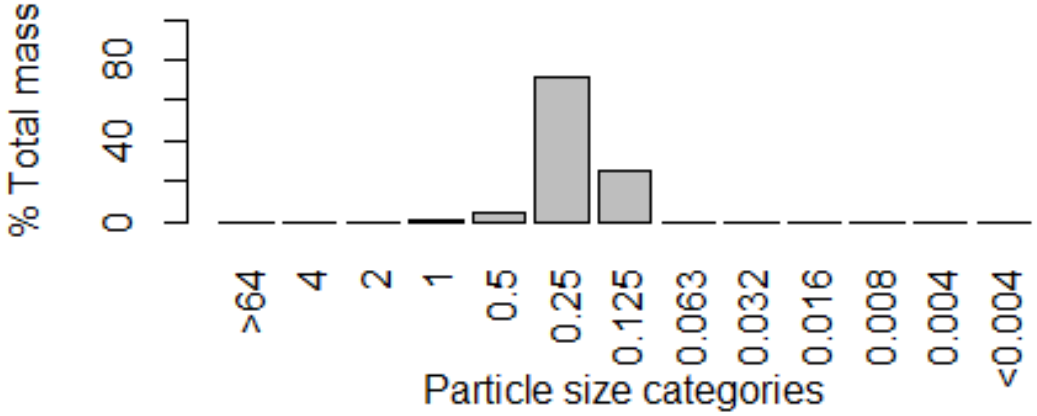


Figure C2. **Bar plot illustrating numbers of *Gammarus pulex* consumed during 24 hours.** Bars represent mean (±1SE) numerical consumption of prey by *Cottus gobio*.

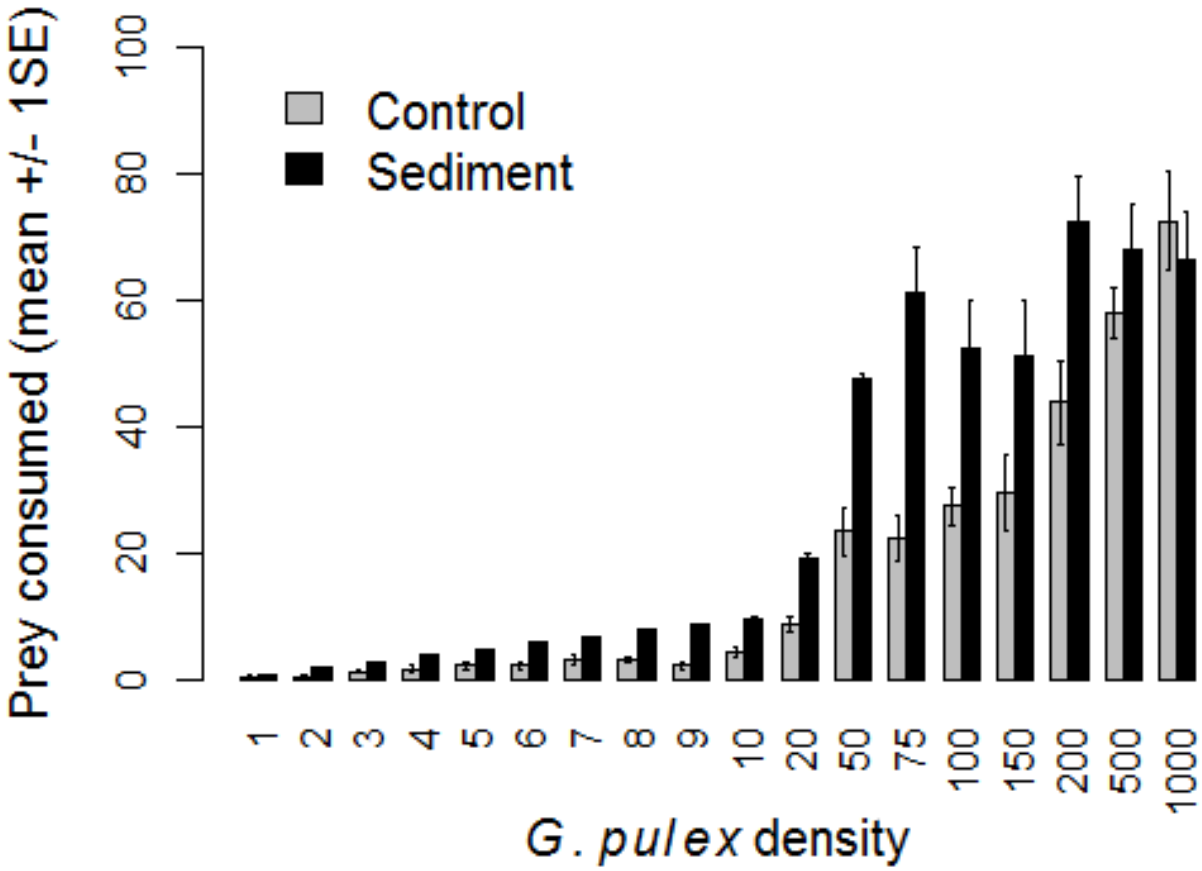
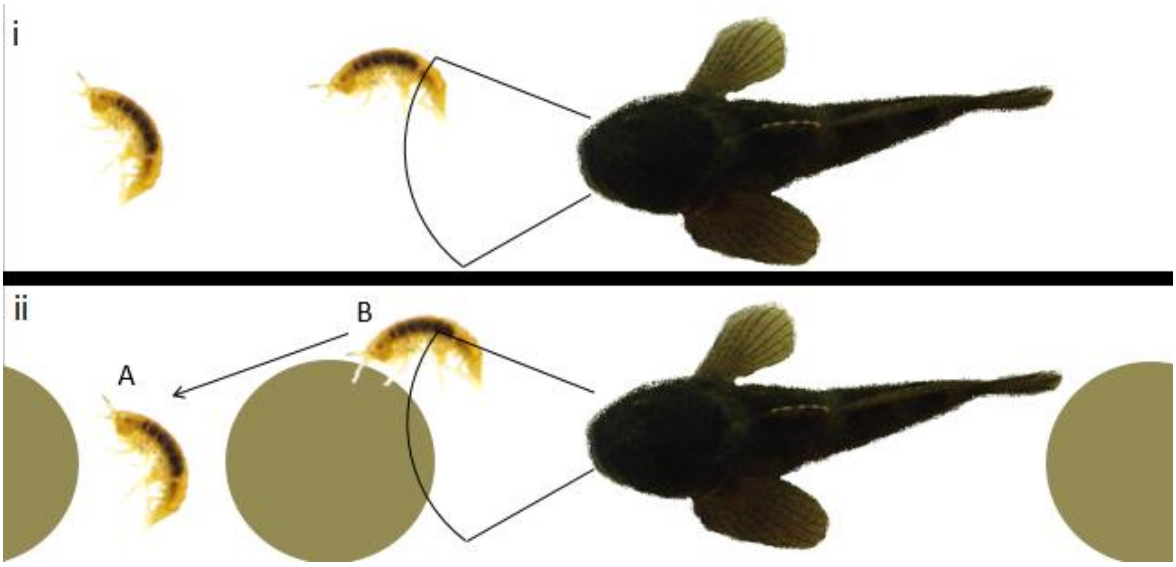


Figure C3. **Schematic illustrating the effect of habitat complexity on predator-prey encounter rate.** Showing i) Sedimented habitat where predator-prey encounter rate is increased, attack rate is increased and attack success is increased (limited prey escape) and ii) control habitat where prey can utilise interstitial space to escape detection (A) and to avoid capture when detected (B to A). Area in front of fish illustrates the predator's visual field.



APPENDIX D

Supplementary material to accompany Chapter Five.

Table D1. **Phenotype descriptors observed during the warming activity threshold experiment.** Pre-heating represents activity during the acclimation to the arena prior to warming ramp commencement.

Genus	Species	Pre-heating	Loss of coordination (CTmax)	Heat Coma	Post Heat Coma
Helobdella	*stagnalis*	Movement in a forward direction using both anterior and posterior suckers.	Jerky movements on the spot, unable to attach to surfaces using suckers.	Final twitch of body.	Individuals usually lateral or dorsal side upwards. If the former then usually curled, slowly straightening.
Tubificidae		Moving awkwardly around arena, occasionally tightly coiling when disturbed by another individual.	Movement rapid but seemingly unable to move from the spot, with some individuals coiled.	Final twitch of anterior end of the tightly coiled body	
Asellus	*aquaticus*	Walking along bottom and sides of arena, stopping occasionally.	Individuals' roll over facing ventral side upwards, and all movement is confined to the spot.	Final twitch of a leg or antenna.	Legs retract inwards.
Gammarus	*pulex*	Individuals swim around the arena within water column, occasionally stopping and remobilising upon being knocked by others.	Following clumsy rapid bursts, individuals sink to the bottom of arena, or float to top, movement confined to the spot, or in circles.	Final twitch of a leg or antenna.	Usually always, individuals lost their hunched shape and the abdomen stretches out, forming straight line along the dorsal edge from anterior end to posterior end
Elmis	*aenea* (adult)	Walking clumsily around the arena, sometimes 2+ individuals attempting to grip one another.	Confined to the spot on the arena floor (often ventral side upwards), or floated to surface, rapid leg movement continues.	Final twitch of a leg.	

Table D1 continued. **Phenotype descriptors observed during the warming activity threshold experiment.** Pre-heating represents activity during the acclimation to the arena prior to warming ramp commencement.

Elmis	*aenea* (larvae)	Gripped onto twig placed on arena floor, occasionally walking up and down the length of the twig.	Loss of grip with twig, falling to arena floor. Failed attempts walking result in movement confined to the spot. Abdominal spasms.	Final abdominal twitch/ leg if visible.
Limnius	*volkmari* (adult)	Walking clumsily around the arena, sometimes 2+ individuals attempting to grip one another.	Confined to the spot on the arena floor (often ventral side upwards), or floated to surface, rapid leg movement continues.	Final twitch of a leg.
Limnius	*volkmari* (larvae)	Individuals walked around the perimeter of the arena.	Individuals wriggled and movement mainly by rolling sideways rather than coordinated walking.	Final twitch of leg, or of tip of abdomen.
Anopheles	*plumbeus*	Individuals floated beneath the water's surface, posterior end of abdomen towards arena edge, head pointing towards centre of arena.	Individuals either sink to bottom or float, rotating on water's surface with heat current. No attempt to resume initial positioning.	Final twitch of abdomen.
Ceratopogoninae		Resting on arena floor, twisting abdomen, occasionally swimming within water column.	Individuals sink to the bottom of the arena, or float at the surface, movements confined to the spot.	Final twitch of abdomen.

Table D1. **Phenotype descriptors observed during the warming activity threshold experiment.** Pre-heating represents activity during the acclimation to the arena prior to warming ramp commencement.

Macropelopia		Crawling around the arena, occasionally resting on the spot.	Crawling ceases, jerky movements on the spot prevail including rolling.	Final twitch of abdomen.	
Micropsectra		Fairly continuous crawling along arena floor, and around arena perimeter.	Crawling ceases, jerky movements on the spot prevail including rolling.	Final twitch of abdomen.	
Dicranota		Move around bottom of arena by means of wriggling abdomen side to side.	Movement confined to the spot on the arena floor, often lateral or dorsal side upwards.	Final twitch of posterior end of abdomen.	
Tipula (*Arctotipula*)		Individuals crawled around the arena floor, stopping occasionally.	Individuals no longer crawl nor contort their abdomen, movements only small twitches, abdomen remains fairly straight.	Final twitch of posterior end of abdomen and/or spiracles.	
Baetis	*rhodani*	Individuals cling onto sides of arena, occasionally swimming in a darting manner between positions.	After rapid bursts around the arena, individuals float or sometimes sink to bottom, often ventral side upwards.	Final twitch of leg.	Legs retract inwards into the body.
Caenis	*luctuosa*	Individuals walk along the bottom of the arena, stopping occasionally.	Movement confined to the spot, or walking in circles.	Final twitch of abdomen, leg or antenna.	

Table D1. **Phenotype descriptors observed during the warming activity threshold experiment.** Pre-heating represents activity during the acclimation to the arena prior to warming ramp commencement.

Ephemera	*danica*	Individuals remain quite motionless on the bottom, occasionally walking along the arena floor.	Movement is confined to the spot on the arena floor, individuals roll over facing ventral side upwards.	Final twitch of leg, or gill.	
Heptagenia	*sulphurea*	Individuals cling onto sides of arena, occasionally swimming in a darting manner between positions.	Individuals lose grip and fall to the arena floor, often facing ventral side upwards.	Final twitch of leg or gill.	Legs retract inwards into the body.
Serratella	*ignita*	Individuals cling onto sides of arena, occasionally swimming in a darting manner between positions.	Individuals lose grip and fall to the arena floor, often facing ventral side upwards.	Final twitch of leg, or abdomen.	Legs retract inwards into the body.
Sialis	*lutaria*	Individuals continuously walk around the arena, occasionally curling up upon being disturbed by another individual.	Individuals often float to surface, with movements confined to the spot, or remain on the arena floor, often turning ventral side upwards.	Final twitch of leg or abdomen.	Legs fold inwards slightly.
Ancylus	*fluviatilis*	Individuals slowly move around the arena, over the arena floor and along the arena sides.	Individuals lose grip of the arena sides and fall from the water's surface to the bottom of the arena, unable to right themselves.	Final twitch of body.	

Table D1. **Phenotype descriptors observed during the warming activity threshold experiment.** Pre-heating represents activity during the acclimation to the arena prior to warming ramp commencement.

Lymnea	*peregra*	Individuals slowly move around the arena, over the arena floor and along the arena sides.	Individuals lose grip, the shell often tips over onto its side, occasionally completely over; the snail's body continues moving.	Final twitch of the body.	
Caolpteryx	*virgo*	Individuals walk around the arena floor, occasionally stopping and sometimes gripping to the sides of the arena.	After some occasional sharp twists of the abdomen, individuals stop walking and often roll over, ventral side upwards. All movements confined to the spot.	Final twitch of a leg or posterior end of abdomen.	Legs retract inwards into the body, and the gap between caudal lamellae closes.
Leuctra	*fusca*	Individuals walk around the arena floor, stopping occasionally.	Individuals often float to the surface, but occasionally stay on the arena floor, all movement confined to the spot.	Final twitch of leg or abdomen.	
Nemurella	*picteti*	Individuals cling onto arena sides, occasionally walking along sides and over arena floor.	Individuals stop walking around arena, often fall over with lateral side facing upwards; occasionally float.	Final twitch of leg.	Legs retract inwards.

Table D1. **Phenotype descriptors observed during the warming activity threshold experiment.** Pre-heating represents activity during the acclimation to the arena prior to warming ramp commencement.

Agapetus	*fuscipes*	Individuals occasionally walk around the arena, some climb the arena sides.	Individuals stop walking, and all movement is confined to the spot. Some individuals leave their case.	Final twitch of leg or abdomen.	
Drusus	*anulatus*	Continuously walking around arena floor.	Walking ceases and individuals fall to face ventral side upwards. Movement confined to spot. Individuals never leave case, but rarely retract in either.	Final twitch of leg.	
Hydropsyche	*pellucidula*	Individuals walk around arena floor, occasionally contorting body when disturbed by another individual.	Walking around the arena ceases, all movement confined to the spot.	Final twitch of leg or abdomen.	Body curls up, with posterior end of abdomen bending towards the head.
Rhyacophila	*dorsalis*	Individuals walk around arena floor, occasionally rolling across the floor when disturbed by another individual.	Individuals stop walking around the arena, and all movement is confined to the spot.	Final twitch of leg or posterior tip of abdomen/anal proleg.	Body curls up, with posterior end of abdomen bending towards the head.
Polycelis	*nigra*	Individuals continuously swim around the perimeter of the arena floor, and along the arena sides.	Individuals begin to lose grip with the arena, and begin to contort their body in a spiralling ribbon like manner.	Final twitch of body.	Rather soon after final movement, individuals start to lose their body shape, changing colour and swelling up and rapidly breaking down.